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THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS

IV. THE RESPONSE OF THE HUMAN STOMACH

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THE term "response," as used in the title, was introduced by Bergeim, Rehfuss and Hawk (1919, 1920) and their associates to signify the effects of food on both the secretory and motor functions of the stomach. These authors employed in the course of their studies a great variety of foods but did not include the breakfast cereals. The object of this portion of our general survey of these foods was, first, to observe whether whole grain cereals affected the stomach differently from endosperm of grain, and, second, whether stomachs of different individuals respond similarly. Incidentally, it was desired to compare the several cereals as test meals.

For gastric analyses a great variety of test meals has been employed, the most common one being the Ewald meal of white bread (or toast) and tea. Rehfuss and Hawk (1921) advance good reasons for adhering to this meal for routine clinical examination of stomach contents, even by the fractional method. Another favorite meal for study of the acidity curves by the fractional method has been the oatmeal-gruel meal originally introduced by Boas. Two tablespoons of oatmeal are placed in a quart of water and the mixture boiled down to a pint. It is then strained, seasoned with salt and drunk rapidly while warm (Crohn and Reiss, 1917). Nearly all such test meals employ cereal products of some sort. Strauss (1907) used a fat-containing zwieback, and Dock (See Gorham, 1921) introduced our familiar Shredded Wheat biscuit. Test meals moreover are usually taken early in the day on empty stomach and are therefore properly called test breakfasts. The practical considerations in the choice of a test breakfast are: 1, that it be so constituted that the gastric contents (or samples thereof) can be readily recovered at different intervals; 2, that a filtrate

devoid of colors which might interfere with the titrations be readily obtained; and 3, if acidity curves and pepsin curves be desired, that the meal be of such a nature that the stomach contents at different intervals are homogenous or can be made homogenous by simple procedures. Gorham (1921), Wheelon (1921), Kopeloff (1922) and White (1924) have demonstrated that great variations in acidities at different levels of the gastric contents may be obtained unless very special precautions are taken to mix the contents thoroughly before withdrawing a sample. Gorham, indeed, believes "that in order to speak of quantitative gastric analysis, the stomach must be emptied completely at a definite time," and "the true fractional analysis necessitates the giving of successive test meals and extracting them at different periods." The same procedure is advocated by Jones (1924). From this standpoint a thin gruel (the thinner the better), a solution of peptone, the caffein stimulating solution of Katsch (1927), twenty per cent alcohol, or just plain water, is probably the best type of "meal" to use. The question may fairly be raised whether a test breakfast intended for the study of secretion and motility of the stomach should not bear some resemblance to the breakfast which the subject is accustomed to take. Tea is very rarely taken for breakfast in this country and cereal gruels are practically never taken without milk or cream and sugar. The gruel meal of Boas without other seasoning than salt is admittedly (Bennett and Ryle, 1921) a very insipid meal and the Ewald meal of bland toast and tea is little better. The pleasure of eating must have some effect on the psychic secretion of gastric juice. Both tea and coffee interfere somewhat, on account of their characteristic colors, with the color changes of indicators commonly used in determination of the acidities, in the early fractions; but the coffee, and to a large extent the cream taken with the cereal, should be evacuated from the stomach by the end of the first hour and perfectly satisfactory end-point reactions should be obtained after this interval. Furthermore, certain careful studies (Wheelon, 1921; Bell and MacAdam, 1924) indicate that the widest variations in the acidity values of normal stomachs occur more particularly in the early fractions and much more uniform values after the one-hour interval. Hence unless there is some special reason for examining the stomach contents less than one hour after eating, the ordinary cereal breakfast food with coffee, sugar and cream as used so commonly in the American family should prove a more satisfactory test breakfast in the great majority of cases.

Is one of the most frequently eaten cereals better than another? The present study offered the opportunity of making some controlled observations bearing on this question. How far can the emptying time of the

stomach with a test meal be taken as a measure of digestibility of the food eaten? The second question must be answered first.

Since the several cereals as prepared for consumption as breakfast foods have much the same composition (Murlin, Line, Piper and Pierce, 1929), the rapidity with which each can be disposed of by the stomach should depend chiefly upon the state of subdivision conferred upon it by the process of chewing. The process of manufacture might leave one food rather coarse, another very finely divided; but if the former were precooked or cooked longer just before eating, it might prove the more digestible in the stomach, assuming that the two were chewed and insalivated to the same extent. Since the classical study of Beaumont (1833) we have known that easily digested and well-cooked foods leave the stomach early, while relatively indigestible and poorly cooked foods remain longer. More modern studies indicate that any unusually irritating material tends to delay the passage of food into the stomach (Moritz, 1901; Carlson, 1916). Mattill and Smith (1930) have shown in the study on rate of digestion and absorption of the same cereals in the rat that physical consistency as conferred by cooking, rather than the presence of roughage in the form of bran, seems to determine the length of sojourn in the stomach of that animal. By cooking the same length of time just before eating and controlling the manner of eating it should be possible then to judge the "digestibility" in the stomach to some extent by finding how long the food remains in the stomach or how much of it remains after a given length of time.

Adequately controlled comparisons therefore would require: 1, that the foods as taken from the packages be cooked, if cooked at all, in identically the same way; 2, that they be taken in equivalent amounts and be eaten in conjunction with the same other foods in the same amounts; 3, that they be chewed the same length of time; 4, that they be eaten successively by the same person or persons; 5, at the same interval since the last meal; and finally, 6, that the stomach contents be removed at the same intervals after eating.

All these conditions were complied with in both the studies about to be described. The two studies differed somewhat in the method of ascertaining the effect on stomach action. In both, the test meals were eaten with the stomach tube in place. In the first study the stomach contents were withdrawn and the residue washed out with a small quantity of water (150 cc.) two hours after eating. In the second study the intervals after eating when the contents were withdrawn varied from thirty minutes to three hours, but were the same for any given individual with all the cereals.

The amount of water used in washing at the earlier intervals was necessarily much greater.

The First Study

Procedure. Three subjects were used in this study, all of them members of the staff of investigators. They weighed respectively 214, 151 and 148 pounds. They came to the laboratory without breakfast and, as a rule, having the stomach tube in place. In a few instances the tube was swallowed after the subject came to the laboratory.

The Rehfuss (1914) tube was used in all of the tests. In taking the tube an effort was made to use only as much water as necessary to facilitate its passage. When more than one or two swallows of water were found necessary, sufficient time was allowed, before taking a sample of the gastric juice, or before introducing the meal, to permit the water to leave the stomach by way of the pylorus. Ordinarily this requires only a few minutes.

Two sets of observations were made—one on the acidity of the gastric contents and the other on the rate of disappearance of the protein of the test meal from the stomach. For the first set it was necessary to take a sample of the fasting or hunger juice, as a starting point. This was usually obtained by 8:30 a.m., the subject not having eaten or taken water since the previous evening. The stomach was washed by passing a known quantity of distilled water through the tube and immediately withdrawing it. Then the meal was eaten with the tube in place, after which the subject went about his laboratory work, the end of the tube being clamped to prevent siphoning of the stomach contents.

Test Meals. One test meal of each of the three cereals, Wheat Endosperm,[1] Toasted Corn Endosperm and Regular Oats, was taken by each subject. The meals consisted of

Dry cereal	25 grams
Water	200 cc.
Cane sugar	50 grams
Cream (20%)	80 cc.
Coffee	170 cc.

This made an ordinary serving and was taken with cream and sugar and with coffee, as such cereals are usually taken by the average American consumer. The Wheat Endosperm and Regular Oats were cooked in a double boiler for twenty minutes, the Toasted Corn Endosperm was merely soaked in the cream and water.

Nitrogen Determinations. In carrying out the Kjeldahl method on a meal or on stomach contents, it was necessary first to thoroughly homogenize the mixture by a preliminary partial digestion. This was done by boiling the entire mass with 1/10th its volume of concentrated sulfuric acid for two hours, after which, and thorough stirring, duplicate representative samples could be obtained for the regular Kjeldahl method.

Titration of free and total acidity. Ten cubic centimeters of filtered gastric contents were titrated by the usual clinical method, using Töpfer's reagent for titrating the free HCl and phenolphthalein for titrating the total acidity. The acidity of the gastric contents was then expressed in terms of N/10 HCl per 100 cc. of gastric contents.

Results: Digestion having gone on for two hours, the entire stomach contents were removed by means of a large syringe, water being introduced to wash out the last traces of the food. During the removal the subject assumed all possible positions, standing, lying on side, back, abdomen, etc. in order that the bucket should find all depressions of the lining. The subject aided also by contractions of the abdominal muscles, thereby shifting

[1] See paper I, *This Journal*, II, 83, for description of the several cereals used.

the position of the bucket to and fro. Usually 150 cc. of distilled water introduced through the tube was enough to wash out the last traces of food particles left at two hours. The entire volume of chymified food with the washings was then assembled, measured, and thoroughly mixed, a portion filtered and analyzed for acidity and another portion for nitrogen by the Kjeldahl method. A meal entirely similar to the one eaten was similarly analyzed for nitrogen. The difference in nitrogen content expressed as a percentage tells how much of the meal had been disposed of by the stomach in two hours. The results are given for the individual subjects in Table I.

The acidity figures, expressed in terms of 100 cc. original gastric contents, are given in Table I for the fasting stomach and for the contents after digestion for two hours. They are significant only as showng that the subjects used were entirely normal with respect to gastric digestion.

Referring to Table I it is seen that with subject I, 90 per cent of the meal had been disposed of in two hours in the case of the Toasted Corn Endosperm and Wheat Endosperm, but that only 78 per cent of the meal had been evacuated in the same time in the case of the Regular Oats. With subject II the Corn Endosperm was the least readily disposed of (71 per cent), while there was agreement between Wheat Endosperm and Regular Oats (83 per cent). Subject III showed nearly complete emptying of the Wheat Endosperm (97 per cent), but considerably less (85 per cent) of the other two. The Wheat Endosperm is at the highest percentage obtained with all three subjects. Neither of the other two cereals excels it for "digestibility" in the stomach in any one of the subjects.

Discussion

The acidities obtained at two hours after ingestion are well within the normal range as determined by Rehfuss and Hawk (1914), and Bell and MacAdam (1924) for the Ewald meal, and by Crohn and Reiss (1918) and Bennett and Ryle (1921) for the gruel meal of Boas. There was no difficulty with the end points in titration. The meals were flavored to suit the individual tastes of three subjects and were eaten with relish, notwithstanding that the retention tube was in place. By keeping the rubber tube between the cheek and teeth, it was possible to chew the food thoroughly and insalivate it as much as if the tube were not used. None of the subjects was nauseated by the tube. Salivation was not excessive and it was therefore not considered necessary to expectorate what was produced after the meal was ingested. Bell and MacAdam (1924) state that swallowing the saliva while the tube remains in place after eating makes very little difference in the acid curves unless they are distinctly of the low acidity type.

TABLE I
RESPONSE OF STOMACH 2 HRS. AFTER EATING CEREAL FOODS

Subj.	Gastric Acidity cc. N/10 HCl per 100 cc.				Total Nitrogen (grams)			% of Protein Emptied	
	Free		Total		In test meal	In stom. cont. after 2 hrs.	Diff.		
	Fasting	After 2 hrs.	Fasting	After 2 hrs.				Indiv.	Aver.
	Toasted Corn Endosperm				Toasted Corn Endosperm				
I	1.0	38.0	5.0	50.0	0.588	0.058	0.530	90.0	
II	2.0	26.0	5.0	39.0	0.588	0.173	0.415	71.0	82
III	7.0	66.0	10.0	82.0	0.588	0.087	0.501	85.0	
	Wheat Endosperm				Wheat Endosperm				
I	10.0	54.0	12.0	68.0	0.688	0.069	0.619	90.0	
II	7.0	27.0	12.0	48.0	0.688	0.128	0.560	83.0	90
III	23.0	28.0	27.0	36.0	0.688	0.020	0.668	97.0	
	Regular Oats				Regular Oats				
I	10.0	50.0	14.0	67.0	0.721	0.159	0.562	78.0	
II	7.0	66.0	10.0	82.0	0.721	0.124	0.597	83.0	82
III	14.0	63.0	17.0	79.0	0.721	0.111	0.610	85.0	

Complete acidity curves were not obtained because, as proved by Gorham, Wheelon and especially White, quoted above, this necessitates mixing thoroughly the stomach contents for each sample. The conviction had already been reached that acidity curves are more reliably obtained with simple stimulating solutions.

The first study was merely a preliminary one for determination of the relative rates of emptying the three kinds of cereals used. The slightly more rapid average emptying for the three subjects, of the Wheat Endosperm product cannot be accepted as conclusive because of the small number of tests.

The Second Study

The second study was planned expressly to test the emptying time for a pure endosperm product without roughage, as compared with others consisting of the whole grain.

Procedure. Four subjects were used, all of them laboratory workers and all but one engaged upon the cereal investigation. Their respective weights were 215, 154, 136 and 181 pounds. All were in good health though not at all in muscular training. The Rehfuss stomach tube was used in the same manner as before except that it was introduced always after the subject came to the laboratory, without the use of water, and just before eating the meal. No food had been taken since the night before and gastric contents were drawn just before eating to confirm the absence of food.

The routine was so planned that one subject ate the same cereal on four or five successive days of the week and submitted himself for evacuation of the stomach by the stomach tube at progressively longer intervals after eating each successive day. For example, on the first day the stomach was emptied at the end of one hour, the next day after one-and-one-half hours, etc.

During this time the food was cooked, on the several days, the same length of time. The next week the subject ate another cereal cooked the same length of time and as before submitted to evacuation of the stomach contents after the lapse of one-half hour, one hour, one-and-one-half hours, and two-and-one-half hours, on successive days.

Both acidity and nitrogen determinations were made just as in the earlier study but the acidity figures are not reported. They show only that the subjects were normal as regards free and total acidity in the gastric contents.

It was soon learned that the amount of food which had been evacuated from the stomach into the intestine one-half hour after eating was very small and very variable; hence the results were discarded. Likewise, it was found to be unnecessary, except in the case of one subject, to evacuate the stomach more than two-and-one-half hours after eating, for the meal was small enough that all the food, on several occasions, had been emptied within this time. It was better to find some food than to find the stomach empty, for in the latter case there was no telling how long it had been empty.

The composition of the meals was slightly different for the different subjects as may be seen from the following table for the first week of the experiment.

Two subjects took coffee and two drank water with the meals. The same amount of fluid and of each of the other ingredients of the meal was taken each day except that subject IV found 40 grams of sugar too much after a few days and changed to 30 grams. Since carbohydrate was not being determined, this would make no difference in the results on the amount of food emptied at different intervals, judging by the nitrogen (protein).

TABLE II
TEST MEALS USED

Subj.	Cereal*	40% Cream	Sugar	Coffee	Water	Cooking time for cereal
	gm.	cc.	gm.	cc.	cc.	min.
I	25	40	40	170	none	5
II	25	40	30	none	100	15
III	25	40	30	none	60	30
IV	25	40	40	100	none	60
			—			
			30			

* 2/3 cup of hot water and 1/4 teaspoonful of salt added to the 25 gm. of dry cereal in top of double boiler. Cooked over boiling water for the time indicated.

Table III shows the nitrogen content of the different meals. At the most, only 18 per cent of the nitrogen was in the form of other ingredients than the cereal itself. This occurred with Wheat Endosperm where the cereal furnished 0.430 grams N, the cream 0.011 grams and 170 cc. of coffee 0.081 grams. The smallest amount furnished by non-cereal food was approximately 11 per cent

TABLE III

	Wheat endosperm	"Whole wheat"	Precooked oats	Regular oats
% N	1.72	1.95	2.50	2.26
Amt. N in 25 gm. cereal	.430 gm.	.488 gm.	.625 gm.	.565 gm.
Cream 0.28% N 40 cc.	.011 gm.	.011 gm.	.011 gm.	.011 gm.
Cereal plus cream N =	.441 gm.	.498 gm.	.636 gm.	.576 gm.
Coffee (0.047% N) N in 100 cc.	.047	.047	.047	.047
In 170 cc.	.081	.081	.081	.081
N in meal without coffee	.441 gm.	.499 gm.	.636 gm.	.576 gm.
N in meal with 100 cc. coffee	.489 gm.	.546 gm.	.684 gm.	.624 gm.
N in meal with 170 cc. coffee	.522 gm.	.580 gm.	.717 gm.	.657 gm.

and occurred with the Precooked Oats where the cereal furnished 0.625 grams N, the cream 0.011 grams, and 170 cc. coffee 0.081 grams. It was thought better to tolerate the small error due to the difference between 11 and 18 per cent of non-cereal nitrogen and keep the relative total weights of cereal, cream, coffee, etc. the same, than to equalize the nitrogen and alter the ratio of total cereal, cream, etc. The difference comes about because of the greater percentage of nitrogen (protein) in the oats.

A further improvement in the method adopted in this second study consisted in the much more thorough rinsing of the stomach in removal of the contents. With the coarser cereals particularly, and at the shorter intervals after eating, it was found necessary to use a much larger volume of water, than had been used in the first study, in order to remove the last trace of cereal from the stomach. Volumes as high as 1500 cc. and in a few instances two litres, were

used in rinsing out the last traces of the meal at the earlier intervals. In the process of removing the stomach contents with the subject sitting upright, it was frequently noted that there was a distinct stratification of the several ingredients composing the meal. The cereal grains were found in the lowermost part of the stomach and cream and coffee, where coffee was used, were found in a layer above these. This was true in evacuating the stomach one-half hour and one hour after eating, but beyond that point most of the fluid contents had, as a rule, been evacuated.

Results: The results are brought together in Table IV which exhibits the several cereals compared with one another horizontally and the several subjects compared with one another vertically in the table. For example, the four subjects showed with Wheat Endosperm at the end of one hour that 32, 44, 22, and 40 per cent respectively of the stomach contents had been evacuated into the intestine. At the end of two hours 81, 87, 45, and 100 per cent respectively had been evacuated into the intestine. Comparing the different cereals for subject I, it is found that at the end of two hours Wheat Endosperm showed 81 per cent evacuated, "Whole Wheat" 86 per cent, Precooked Oats 74 per cent, and Regular Oats 78 per cent. At the bottom of the table the averages of the several subjects for each cereal are shown so far as they can be compared. A few irregularities crept into the table for unavoidable reasons; for example, instead of stopping digestion at the end of two and one-half hours exactly, in the case of subject I on Wheat Endosperm, the stomach was not evacuated until two and three-quarters hours; also with subject IV on Precooked Oats, instead of evacuating the stomach at one and one-half hours exactly, the time had gone to one and two-thirds hours. These slight differences do not affect the average results materially. On the average, Wheat Endosperm at the end of one hour had been disposed of by the stomach to the extent of 35 per cent and exactly the same figure appears in the average for "Whole Wheat," while for Precooked Oats the average is considerably better; namely 44 per cent, and for Regular Oats slightly worse; namely, 34 per cent. At the end of two hours, however, Wheat Endosperm catches up with Precooked Oats and passes the other two cereals, showing an average of 78 per cent disposed of by the stomach as compared with 68 per cent for "Whole Wheat" and 71 per cent for Regular Oats. The comparison at two and one-half hours is incomplete and is only estimated for "Whole Wheat." This was done in the case of subject III by taking the half-way point between two hours and three hours and for subject IV by extrapolating beyond the two-hour point by taking the general direction of the curve shown in Chart No. 1. While the results as exhibited by the table indicate a clear advantage on the part of Wheat Endosperm over the other cereals, with the exception of precooked Oats, at the end of two hours and two and one-half hours, the difference is not very great. As already mentioned above, the peculiarity

TABLE IV

EMPTYING RESPONSE OF THE STOMACH AT DIFFERENT TIMES AFTER EATING CEREAL FOODS

Subj.	Time in stomach hrs.	Precooked Oats				Regular Oats				Wheat Endosperm				"Whole Wheat"			
		Tot. N meal gms.	N in stomach gms.	Emptied gms.	%	Tot. N meal gms.	N in stomach gms.	Emptied gms.	%	Tot. N meal gms.	N in stomach gms.	Emptied gms.	%	Tot. N meal gms.	N in stomach gms.	Emptied gms.	%
I	1	0.717	0.415	0.302	42	0.657	0.425	0.232	35	0.522	0.353	0.169	32	0.580	0.324	0.256	44
	1½	0.717	0.274	0.443	62	0.657	0.194	0.463	70	0.522	0.240	0.282	54	0.580	0.185	0.395	68
	2	0.717	0.184	0.533	74	0.657	0.142	0.515	78	0.522	0.102	0.420	81	0.580	0.081	0.499	86
	2½	0.717	0.000	0.717	100					0.522	0.035	0.487	93	0.580	0.036	0.544	*93
	3					0.657	0.027	0.630	96								
II	1	0.636	0.353	0.283	45	0.576	0.386	0.190	33	0.441	0.244	0.197	44	0.499	0.339	0.150	32
	1½	0.636	0.181	0.455	72	0.576	0.300	0.276	57	0.441	0.173	0.268	61	0.499	0.149	0.350	70
	2	0.636	0.059	0.577	91	0.576	0.189	0.387	67	0.441	0.057	0.384	87	0.499	0.132	0.367	74
	2½					0.576	0.000	0.576	100	0.441	0.023	0.418	95	0.499	0.137	0.362	**73
III	1	0.636	0.441	0.195	31	0.576	0.371	0.205	35	0.441	0.343	0.098	22	0.499	0.378	0.121	24
	1½	0.636	0.441	0.195	31	0.576	0.347	0.229	40	0.441	0.335	0.106	24	0.499	0.342	0.157	32
	2	0.636	0.286	0.350	55	0.576	0.251	0.325	56	0.441	0.241	0.200	45	0.499	0.376	0.123	25
	3					0.576	0.153	0.423	73	0.441	0.132	0.309	70	0.499	0.158	0.341	68
IV	1	0.684	0.283	0.401	59	0.624	0.419	0.205	33	0.489	0.294	0.195	40	0.546	0.312	0.234	43
	1½	0.684	0.067	0.617	*90	0.624	0.102	0.522	84	0.489	0.201	0.288	59	0.546	0.142	0.404	74
	2	0.684	0.046	0.638	93	0.624	0.099	0.525	84	0.489	0.000	0.489	100	0.546	0.056	0.490	†90
Av. of all Subjects																	
	hr.																
	1				44				34				35				45
	1½				63				62				50				61
	2				78				71				78				68
	2½												84				81
	3								89								est.

* 1¾ hr.

* 2¾ hr. ** 2 hr. † 2⅛ hr.

of the stomach is of greater moment in determining the exact percentage emptied at a given time than is the nature of the cereal. This comes out much more sharply in the graphs drawn to represent the numerical data given in Table IV, (Chart 1).

Subjects I and II, it will be noted, show the same general rate of evacuation, as indicated by the slopes of the curves. Subject III shows a much slower rate and subject IV a much more rapid rate. Each subject is consistent in exhibiting his characteristic rate on all of the cereals. In Chart 2 are brought together the four curves representing the average evacuation rate of all four subjects for each of the four cereals. It is striking how little these differ amongst themselves. In a general way we can say that at the end of one hour from 35 to 40 per cent of all the cereal protein had been removed from the stomach; at the end of one and a half hours from 50 to 60 per cent; at the end of two hours from 65 to 75 per cent; two and one-half hours 80 to 90 per cent and three hours 90 to 100 per cent.

Taking the average slope of the four curves and projecting it to three hours, it is quite evident that in the average case the stomach would be completely empty in three hours. As a matter of fact Subject IV was found to have an empty stomach at the end of two hours with Wheat Endosperm, Subject I at the end of two and one-half hours with Precooked Oats, and Subject II at the end of two and one-half hours with Regular Oats. It must be noted in this connection that the entire breakfast consisted only of an ordinary serving of cereal with somewhat more than the usual amount of cream, but not more than the usual amount of sugar. Coffee was taken, as already noted, by two subjects namely I and IV and was omitted by Subjects II and III. If there had been any distinctive influence of the coffee one way or the other Subjects I and IV should have exhibited either more or less rapid rates of emptying. It should be emphasized again that the four cereals for any given subject were cooked exactly the same length of time; namely, five minutes for Subject I, fifteen minutes for Subject II, thirty minutes for Subject III and sixty minutes for Subject IV. If there had been any influence of the cooking time upon evacuation time, Subject I should have been distinctly slower and Subject IV distinctly faster than the others. As a matter of fact, Subject IV does exhibit the most rapid rate, but this assuredly is not due to the longer cooking time; for the experiment with Precooked Oats after two hours digestion was repeated, the oats being cooked only five minutes instead of one hour, and yet the amount of food evacuated was somewhat higher than after cooking for one hour.

Putting together the results on response of the human stomach obtained

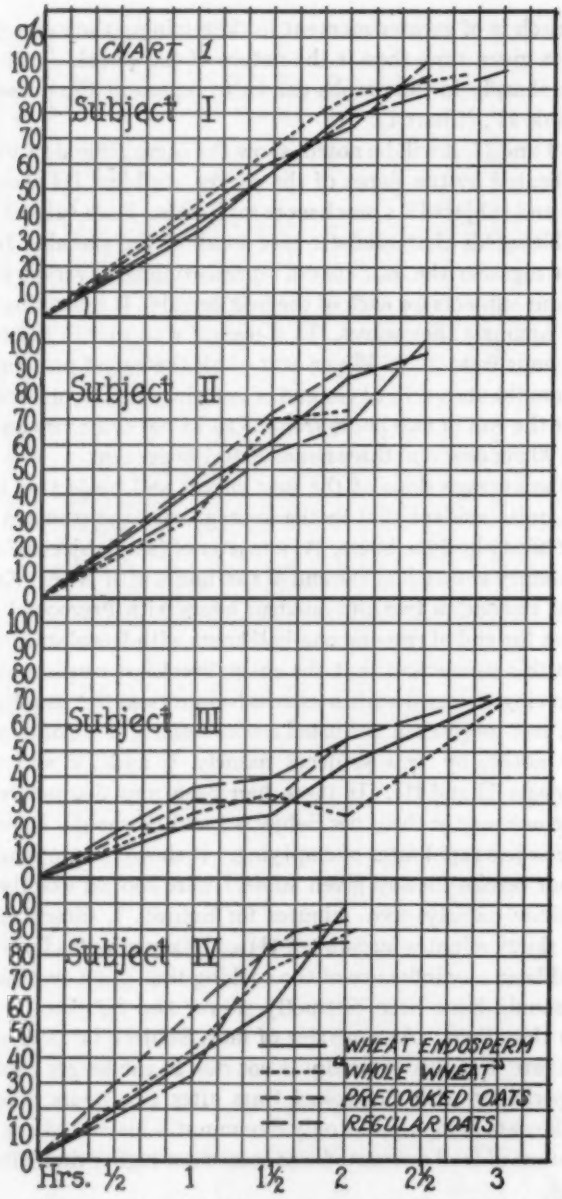

CHART 1. Graphs showing characteristic evacuation rates for four different subjects. Little difference is shown by different cereals in the same subject.

from both studies, we have seven cases which can be averaged for Wheat Endosperm and Regular Oats at the interval of two hours from time of taking food (Tables I and IV). The average percentage disposed of by the

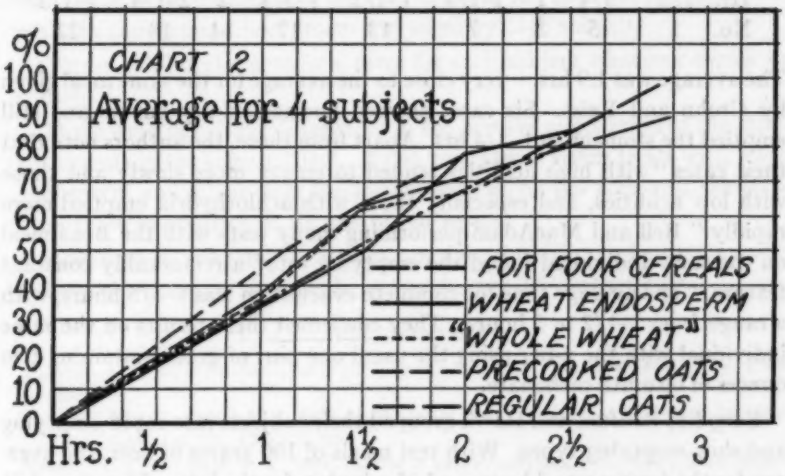

CHART 2. Average evacuation rate for four subjects, for each of four cereals and for all combined.

stomach in this time for Wheat Endosperm is 83, for Regular Oats 76. In only one case of the seven is the oats higher than the wheat product.

DISCUSSION

That the emptying time of the stomach with any given test meal of the sort which may be washed out through the Rehfuss tube can be fairly accurately determined, is the consensus of opinion of the majority of observers who have employed the method. Crohn and Reiss (1917) state that "in hundreds of cases observed with a functionally normal digestive organ the stomach empties itself (of the Boas gruel meal) promptly at two hours." Anderson (1918) asserts that he examined about 800 cases by the fractional method and that "gastric motility can be determined more accurately by the fractional method than by the usual routine roentgen-ray examination." Levy (1918) advocates the use of the Einhorn tube as being "more accurate than the x-ray for gauging the emptying rate of the stomach." Ryle (1920) says of the method "it allows of an estimate of the rate of emptying at least as accurate as that obtained by means of radiography and the barium meal." Bennett and Ryle (1921) however note a marked absence of uniformity in different normal individuals in good

health. They give the following distribution of the emptying time for 100 normal medical students and instructors who had taken the Boas gruel meal.

Hr.	1/2	3/4	1	1–1/4	1–1/2	1–3/4	2	2–1/4	2–1/2
No.	1	5	3	7	13	17	14	18	22

The average was 1.9 hrs.—very close to the average for the same meal given by Crohn and Reiss. Six cases showed normal acidity curves and still emptied the stomach in 1–1/4 hrs. Apart from these, the authors note that their cases "with high acidities tended to empty more slowly and those with low acidities, and especially those with achlorhydria emptied more rapidly." Bell and MacAdam performing many tests with the Boas meal on the same individual found the emptying rate "a remarkably constant feature." The average time for complete evacuation was 1–3/5 hours, with a range from 1–1/2 to 2 hours. They confirmed these results on the same individual with the x-ray using the usual one pint of gruel containing two ounces of bismuth carbonate.

Bergeim, Rehfuss and Hawk grouped their subjects into rapid-emptying and slow-emptying types. With test meals of 100 grams of beef, the average for the former was 2 hours and 35 minutes, for the latter 3 hours and 25 minutes. In the study in their series on gastric response to foods (XII, 1920) dealing with pies, cakes and puddings, they list a number of tests with 100-gram portions of pie crust, crullers, doughnuts, cinnamon buns, spicked cookies, bread and peanut butter, and other articles made principally from wheat flour, and obtain variations of about the same magnitude for emptying time as with 100 grams of beef. Individuals of the slow or rapid type in the main remained true to type on a great variety of tests.

It is quite evident therefore, from the original observations reported in this study, as well as from the numerous other studies reviewed, that the individual response is of greater moment in determining the evacuation time than are small differences in the composition of the test meal.

Summary and Conclusions

Two studies of the gastric response to different cereal breakfast foods in common use in the U.S.A. were made. In the first, three subjects were used and three cereals. In the second, and more complete study, four subjects and four cereals. Each person ate the same cereal on four or five successive days and submitted to evacuation of the stomach contents at successively longer intervals after eating each day. The rate of emptying of the stomach was determined by the amount of nitrogen remaining in the

stomach at each interval and expressing this amount as a percentage of the total nitrogen in the meal as ingested. This procedure was repeated on successive weeks until each subject had eaten all four of the cereals. The test meals were cooked in a uniform manner for each subject and eaten with the same amounts of cream and sugar by each subject.

Curves exhibit characteristic rates for each subject whatever cereal was chosen.

There were but slight differences in the rates of evacuation of the different cereals in the same subject. As an average of seven trials with wheat endosperm and whole oats the former showed 83 per cent and the latter 76 per cent evacuation at the end of two hours.

Roughage in the form of bran in normal proportion or less appeared to influence evacuation time of the stomach very little either way.

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THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS

V. THE BIOLOGICAL VALUE OF SOME CEREAL PROTEINS AS DETERMINED ON RATS

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THE biological value of a protein was defined by Thomas (1) in 1909 as the maximum percentage of the absorbed protein that is usable in anabolism. The original work of Thomas upon himself, and several more recent experiments upon human subjects, notably those of Steck (2), Hindhede (3), Martin and Robison (4), show very considerable divergence in the values assigned to a given protein food and indicate that some unknown variables have been in uncontrolled operation. A critical review of these and other studies on this subject is given in the recent comprehensive book of Mitchell and Hamilton (5). A detailed exposition of the underlying theories and experimental requirements has also been presented by Mitchell (6) and these subjects need not be reviewed here.

The object of this paper is to present a few additional and confirmatory figures for the biological value of cereal proteins obtained by metabolism studies on rats. The technique employed was that perfected by Mitchell (7) for use upon this animal. The only variation was the form of cage, which was similar to the one designed by Ackroyd and Hopkins (8) consisting of a sheet zinc cylinder ten inches in diameter and six inches high, with removable galvanized wire mesh at top and bottom, and resting in a large glass funnel. The provision of food and water and the bulb arrangement for the separation of urine and feces were those described by Ackroyd and Hopkins except that water was supplied in an inverted tube in place of in an open container. By means of cone-shaped sleeves the scattering of food was reduced to a minimum and by the occasional rinsing down of the large funnel with warm dilute acetic acid, a fairly satisfactory separation of uncontaminated specimens was secured.

The cereals examined were Wheat Endosperm,[1] "Whole Wheat," and Precooked Oats. For purposes of comparison a whole milk powder ration, in addition to the necessary basal protein-free ration, was included. The low

[1] These terms are explained in the first paper of this series (9) pp. 85–86.

nitrogen diet was used in preference to the egg diet for a basis of calculation.

The rations were designed to provide the protein on an approximately 5 per cent level, a level at which biological differences are probably most clearly revealed (10). The quantities of finely ground cereals and other constituents which each ration contained are shown in Table I.

TABLE I
PERCENTAGE COMPOSITION OF THE RATIONS USED

	Protein-Free (nearly)	Wheat Endosperm	"Whole Wheat"	Precooked Oats	Milk Powder
Protein source...	0	42.4	41	32	19
Corn starch.....	74	31.6	34	43	55
Agar.....	1	1	0	0	1
Lard.....	18	18	18	18	18
Cod liver oil.....	2	2	2	2	2
Salts*.....	4	4	4	4	4
Vitamin B Harris.	1	1	1	1	1
Nitrogen content.	0.11	0.83	0.86	0.87	0.83

* Osborne & Mendel (11).

The feeding periods were usually 10 days long (in a few cases 7 days) including a preliminary 3-day (or 2-day) period during which excreta were not analyzed. The urine and feces for the remaining 7 (or 5) days were combined into a single sample and nitrogen was determined in duplicate by the Kjeldahl method on aliquot portions, or, in the case of feces, on the total amount. By way of further precaution a given protein feeding period was separated from a non-protein period by not more than one protein-feeding period and the order of the proteins fed to the different animals was not alike with reference to the non-protein periods.

The method of calculation was that developed by Mitchell (7), fecal N of metabolic origin being based on food consumption, and metabolic urinary nitrogen on body weight of the animal.

In Tables II and III are found the average daily nitrogen metabolism data and the calculated biological values for the two series of animals studied. Table IV contains a summary of the biological values obtained from both series; from this table the data questioned in Table III have been omitted.

Data on milk and cereals obtained by others with this method include the following: milk (Mitchell, 10) 18 periods, a range of 81–100 with an average of 93 per cent; whole oat grain (Mitchell, 10) 8 periods, with a range of 75 to 80 and an average of 79; wheat proteins on an 8 per cent

level (Klein, Harrow, Pine and Funk, 12) bran 57, standard middlings 61, red dog 77, second clear 67. That the biological value of a protein as

TABLE II

Rat No.	Body Weight		Food Intake	N Intake	Fecal N	Body N in feces	Food N in feces	Absorbed N	N in urine	Body N in urine	Food N in urine	Food N retained	Biological value
	Initial	Final											
	gms.	gms.	gms.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	Per cent
Low Nitrogen Diet (0.11%) 7 days													
1	225	198	6.7	7	11	1.6†	—	—	56	26§	—	—	—
2	228	201	6.5	7	9	1.4†	—	—	57	26§	—	—	—
3	209	178	6.4	7	11	1.7†	—	—	62	32§	—	—	—
4	199	170	2.9	3	9	3.0†	—	—	48	28§	—	—	—
5	192	168	5.0	5	11	2.2†	—	—	49	27§	—	—	—
Wheat Endosperm 7 days													
2	191	180	8.1	67	17	14	3	64	62	46	16	48	75
1*	201	193	9.1	76	19	20	0	76	67	53	14	62	81
5*	174	167	8.3	69	14	14	0	69	55	34	21	48	70
"Whole Wheat" 5 days													
2	185	180	9.2	79	21	16	5	74	67	44	23	51	69
3	193	182	8.8	75	20?	15?	5	70	63	42	21	49	70
Precooked Oats 7 days													
1	207	198	7.8	68	18	16	2	66	63	54	9	57	86
3	195	181	8.5	74	21	15	6	68	59	48	11	57	84
4*	177	175	8.2	72	21	24	0	72	51	35	16	56	78
Milk Powder 7 days													
4	170	168	8.3	69	13	17	0	69	52	39	14	55	80
5	169	165	8.6	71	19	16	3	68	47	37	10	58	85
Low N Diet (0.11%) 5 days													
1	197	175	6.0	6	14	2.4†	—	—	50	27§	—	—	—
2	182	160	5.8	6	11	1.9†	—	—	41	24§	—	—	—
3	186	172	9.9	11	17	1.7†	—	—	35	20§	—	—	—
4	181	161	3.8	4	11	2.9†	—	—	31	18§	—	—	—
5	171	156	5.8	6	9	1.5†	—	—	30	18§	—	—	—

* 5 day period. † per gram of food consumed. § per 100 gr. body weight.

conceived and determined by this method has a real significance, is attested by the satisfactory agreement between figures obtained by different workers under different laboratory conditions.

TABLE III

Rat no.	Period no.[1]	Body Weight		Food intake	N intake	Fecal N	Body N in feces	Food N in feces	Absorbed N	N in urine	Body N in urine	Food N in urine	Food N retained	Biological value
		Initial	Final											
		gms.		gms.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs	Per cent
First Low Nitrogen Period														
1	0	265	229	7.2	8	—[2]	—	—	—	82	33[3]	—	—	—
2	0	201	177	11.0	12	—[2]	—	—	—	65	34[3]	—	—	—
3	0	182	157	9.2	10	15	1.6[4]	—	—	49	29[3]	—	—	—
4	0	228	205	7.1	8	5	0.7[4]	—	—	74	34[3]	—	—	—
5	0	208	181	5.0	6	6	1.2[4]	—	—	71	36[3]	—	—	—
Wheat Endosperm Periods														
1	1	229	218	9.2	76	21	15	6	70	86	67	19	51	73
2	1	180	181	12.0	100	20	18	2	98	88	60	28	70	72
3	4	152	152	10.2	85	16	14	2	83	65	41	24	59	71
4	4	182	184	10.0	83	22	13	9	74	68	45	23	51	69
"Whole Wheat" Periods														
1	2	225	228	12.0	103	27	19	8	95	88	61	27	68	72
2	5	185	187	11.1	95	23	16	7	88	72	50	22	66	75
4	5	197	194	10.9	94	28	11	17	77	66	44	22	55	71
5	4	176	175	10.5	90	31	15	16	74	59	43	16	58	78
Second Low Nitrogen Period														
1	3	225	207	10.4	11	16	1.5[4]	—	—	52	24[3]	—	—	—
2	3	186	161	7.2	8	10	1.4[4]	—	—	54	31[3]	—	—	—
3	3	169	151	9.3	10	12	1.3[4]	—	—	41	26[3]	—	—	—
4	3	199	180	9.2	10	15	1.6[4]	—	—	50	26[3]	—	—	—
5	3	191	171	8.0	9	15	1.8[4]	—	—	43	24[3]	—	—	—
Precooked Oats Periods														
1	5	219	220	11.8	103	30	17	13	90	70	52	18	72	80
2	4	177	178	11.9	104	25	17	8	96	66	52	14	82	86
3	2	156	171	12.4	108	28	17	11	97	69	44	25	72	74
4	1	206	193	7.6	66	19[5]	8	11	55	67	63	4	51	93[3]
5	2	193	191	11.1	97	29	18	11	86	66	53	13	73	85
5	5	181	181	11.4	100	31	13	18	82	61	46	15	67	82

TABLE III—*continued*

Rat no.	Period no.[1]	Body Weight		Food intake	N intake	Fecal N	Body N in feces	Food N in feces	Absorbed N	N in urine	Body N in urine	Food N in urine	Food N retained	Biological value
		Initial	Final											
		gms.		gms.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.	Per cent
Milk Powder Periods														
1	4	214	209	10.8	90	26	16	10	80	55	51	4	76	95
2	2	188	189	12.3	102	27	19	8	94	71	61	10	84	89
3	5	160	164	11.8	98	24	18	6	92	66	47	19	73	79[6]
4	2	204	204	8.5	71	21	11	10	61	65	59	6	55	91
5	1	185	185	11.3	94	27	15	12	82	64	59	5	77	94
Last Low Nitrogen Period														
1	6	212	198	10.0	11	14	1.4[4]	—	—	47	23[3]	—	—	—
2	6	179	166	9.4	10	12	1.2[4]	—	—	43	25[3]	—	—	—
3	6	155	134	5.3	6	8	1.6[4]	—	—	44	30[3]	—	—	—
4	6	189	179	8.8	10	6	0.7[4]	—	—	38	21[3]	—	—	—
5	6	177	160	9.6	11	7	0.7[4]	—	—	44	26[3]	—	—	—

[1] Since the experimental diets were not fed in the same order with reference to the low N diets, the period numbers have been introduced to indicate the location of each period. The low-N periods for all animals were 0, 3 and 6. All periods were 10 days, with 7-day collections.

[2] Since these determinations were unfortunately lost, the metabolic N in the feces was calculated from the next adjacent low N period.

[3] Per 100 gr. body weight (mean).

[4] Per gram of food consumed.

[5] Inadequate food consumption is probably responsible for loss in weight and apparent high biological value.

[6] Some undeterminable food wastage.

As for the results themselves, it is obvious that oat protein possesses a slight advantage over wheat protein and that the presence in the latter of a small amount of bran does not alter its biological value. Analysis of the

TABLE IV

Protein food	No. of periods	Biological Value	
		range per cent	average per cent
Milk	6	80–95	89
Wheat Endosperm	7	69–81	73
"Whole wheat"	6	69–78	72
Precooked Oats	8	74–86	82

two wheat preparations for fat showed no appreciable difference, indicating that little if any of the germ was present with the bran.

SUMMARY

The biological value of the proteins of three cereal breakfast foods and of milk was determined on rats by Mitchell's method. The proteins were fed on an approximately 5 per cent level. The cereals examined were Precooked Oats, Wheat Endosperm, and "Whole Wheat," with a milk powder ration for comparison. The average biological values obtained from 6 to 8 periods on each food were 89 for milk, 82 for oats and 73 and 72 respectively for the two wheat preparations. The figures are in substantial agreement with those for similar foods obtained by other workers.

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THE COMPARATIVE VALUE OF DIFFERENT FOOD PROTEINS FOR REPRODUCTION AND LACTATION IN THE RAT

II. MILK, EGG AND MEATS

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STRICTLY vegetarian diets have proved themselves to be inadequate to support the normal life cycle of rats. While containing an abundance of carbohydrate and mineral salts, vegetable foods, unless carefully chosen, are likely to provide insufficient amounts of biologically adequate protein, and of the fat-soluble vitamins. With a few exceptions, these important substances are found in greatest abundance in the leafy vegetables and the amount of roughage accompanying them precludes all but the strictly herbivorous animals from consuming adequate amounts. Milk and eggs, because of the excellence of their proteins, and their accessories and mineral salts, are particularly valuable in complementing an otherwise vegetarian diet, without the introduction of meat.

In connection with the experiments on muscle and gland tissue, described in a preceding paper (1930), a study was also undertaken of the relative value of milk and eggs in maintaining the normal life of rats.

Historical

The fact that milk is such an adequate food for the young animal has led many investigators in nutrition to study its properties in detail. Among the well-known workers in this field were Hopkins (1912) and Osborne and Mendel (1913). Mattill (1920) and his coworkers were among the first to test its efficiency for carrying an animal through maturity including reproduction and lactation. He fed rats at first on fresh or powdered milk exclusively but did not obtain normal growth. Neither was there success in reproduction, in spite of the fact that different single additions were made to the milk, including iron citrate, yeast, and ether-extracted wheat embryo.

Daniels and Hutton (1925) secured five generations of healthy young on a diet composed of fresh boiled milk with certain mineral additions. The minerals used resembled those found in soy bean ash, which they found would also supplement whole milk successfully.

Anderegg (1924) fed whole milk powder with supplements of only iron citrate 0.2 per cent, and agar agar 4.8 per cent, and secured some success in both reproduction and lactation. Mitchell (1922) states that agar agar improves reproduction by preventing intestinal obstruction.

The partial success which Anderegg (1924) secured, using supplements of iron citrate and agar agar, may have been due partly to the ash content of the agar agar.

Anderegg and Nelson (1925) made a rather careful analysis of diets containing whole and

skimmed milk powder in different proportions and with various supplements. Many of their statements have been confirmed by more recent work of other investigators. On a diet containing whole milk powder at a 60 per cent level, supplemented by 6 per cent of casein, 0.2 per cent ferric citrate, 4.0 per cent agar agar and dextrin, rats became pregnant and raised young. When 2.4 per cent of McCollum's salt mixture No. 185 was added to this diet, making a total salt content of 6.2 per cent, reproduction and lactation were improved, fourth generation young being raised.

These workers also tested the value for reproduction of Osborne and Mendel's (1913) well known milk food diet, which contained milk powder 60 per cent, starch 12 per cent and lard 28 per cent. Their results were negative. Neither was there success in reproduction on a diet containing 50 per cent of milk powder, 2 per cent salts, 10 per cent lard and starch. This confirms the results of Mattill and Stone (1923). By omitting the lard, adding 4 per cent of agar-agar and substituting dextrin for starch, good results in reproduction and lactation were secured in the first generation and some in the second. When the milk powder was reduced to 30 per cent and supplemented with 20 per cent casein, 3.7 per cent salts, 4 per cent agar agar and 42.3 per cent dextrin, there was fairly good reproduction in the first generation and some success in the second.

According to Evans (1927) milk fat contains very little vitamin E, but the above results would seem to show that in the absence of lard, small amounts of whole milk contain considerable available vitamin E.

Mattill, Carman, and Clayton (1924) fed diets containing 50 per cent milk powder, 15 per cent of lard, 2 per cent salts, cornstarch, and 2 per cent and 5 per cent of other materials containing nucleo-protein, but had no success in securing reproduction until supplements which contain vitamin E, such as wheat embryo or lettuce, were added. These same workers secured good reproduction and fair lactation on a diet containing milk powder 40 per cent, casein 8 per cent, yeast 5 per cent, salts 2 per cent, and starch, but no lard. They did not attempt to explain this difference in results but were convinced that the lard must in some way lessen the availability of vitamin E, or increase the requirement.

The literature contains very few references to experiments in which whole egg was fed. Usually the white or yolk was given alone or with various supplements, and as a rule only the effect on growth or the coefficient of digestibility was studied.

McCollum (1922) states that eggs contain everything that is necessary for the growth and maintenance of the body but are poor in calcium and are unbalanced with respect to certain other food principles. They are rich in fats and related substances and are nearly lacking in carbohydrates.

Experiments have shown that egg yolk is much more adequate than egg white as a sole food. McCollum (1909) fed two young white rats on egg yolk alone for 18 weeks. The eggs were boiled hard and the whites completely separated from the yolks. One rat, a male, gained weight from 71 grams to 167 grams. The other, a female, gained from 40 grams to 160 grams during the same period. Just before the close of the experiment she gave birth to eight young which had developed while the mother subsisted entirely from egg yolk.

In contrast, the results of feeding an exclusive diet of egg white show that it is entirely inadequate for supplying the nutritive requirements of an animal such as a rat (Maignon 1912, von Knieriem 1885). The animal quickly loses weight and if death does not occur too soon, it develops an eye disease, indicating a deficiency in vitamin A.

It appears from the work of a number of investigators that either egg white or egg yolk when properly supplemented furnishes protein adequate for normal growth in the rat. In fact, Mitchell (1924) found the biological value of egg proteins to be 93 with no loss in digestion when fed at an 8 per cent level.

Osborne and Mendel (1913, 1915) fed ovalbumin at an 18 per cent level and obtained normal growth when the protein was supplemented with starch, lard, minerals and vitamins.

Bond (1922) also obtained excellent growth in rats when egg white was given as 20 per cent of the ration, but Boas (1924) using it at the same level, was not equally successful. The fact that

Boas' animals lost hair after 4 to 6 weeks on the diet makes one suspect a vitamin B deficiency, rather than a protein one.

Bateman (1916) found a great difference in the utilizability of egg white, according to whether it was fed raw, cooked, partially digested with pepsin, or after treatment with various chemicals. Raw egg white, when it made up a large percentage of the diet of dogs, rats, rabbits, and man, was found to be decidedly indigestible, producing diarrhea. This result was not noted after treatment in the other ways mentioned. The indigestibility of the raw egg white was thought to be due possibly to its antitryptic effect. Boas (1927) has more recently directed attention to the very different nutritive value of heated and unheated egg white in rats.

Rose and McLeod (1922) fed whites of eggs to human subjects in as large amounts as 10 to 12 whites daily as part of a mixed diet. The average coefficient of digestibility for raw egg white was 80 per cent as compared with 86 per cent for cooked whites in the same diet. Absorption varied with the method of preparation, being less for raw whites in their natural state than when beaten light.

Other workers did not find as good utilization of raw white as this. Falta (1906) got 70 per cent utilization and Wolf (1912) 50 per cent.

Osborne and Mendel (1913) secured normal growth when they fed ovo-vitellin at an 18 per cent level with supplements of starch, lard, minerals and vitamins.

McCollum and Davis (1913) found the fats of egg yolk fully comparable with butter fat as a source of vitamin A and Osborne and Mendel (1923) state that egg yolk is a fairly good source of vitamin B, 1.5 grams of the fresh yolk being required daily to supply a 100 gram rat.

McLester (1927) states that egg yolk also contains vitamin D. He says that calcium, phosphorus and iron are found chiefly in the yolk and that the yolk fat equals that of milk, being superior to most other forms, in that it is digested to a large extent in the stomach.

Experimental

Rations. As in the meat feeding tests, the rations containing milk and egg were devised to supply protein at a level of 15 and 20 per cent, together with necessary supplements of carbohydrate, fat, salts and accessories Fat, when needed, was supplied by lard, carbohydrate by cornstarch, and vitamins A and D by cod liver oil. The salt mixture used was that of Osborne and Mendel (1917). The dry milk used was a whole milk powder supplied by the Merrell-Soule Co., the egg was a whole egg flake supplied by a local dealer. The manufacturer provided the analyses of whole and skim milk powder, which, together with the average of several analyses of the egg flake, were as follows:

	Protein	Fat	Ash
Whole milk powder.....	26.67	28.20	5.75
Skim milk powder.....	37.70	1.35	8.21
Egg flake.....	43.75	42.46	3.50

The rations were compounded and stored in the manner described for the meat rations and their composition was such as to parallel as closely as possible the diets containing meat proteins.

Series I—Moderate Protein, High Fat

The first series was planned as follows:

Composition of Rations
(15% protein, 20% fat, 5% ash)

Ration No.	Product (powdered)		Starch	Lard	Salts	Fe Citrate	Yeast	c.l.o.
100	Milk	56.8	32.1	2.0	2.0	0.1	5.0	2.0
110	Egg	34.2	51.3	3.5	4.0		5.0	2.0

Milk, Ration 100. Six females and 4 males were placed on this ration. Growth was rather variable in the females, being normal or above in 4 and below normal in 2 at 100 days of age, which is about the beginning of the reproductive period. At this age the males had all shown normal growth.

At the age of 125 days only 3 of the females had borne one litter each. Lactation was successful in the case of only one female that raised 4 out of 5. A shortage of vitamin E was plainly evident, so at this point 2 per cent of wheat embryo oil was added to the ration. Following this, all of the females responded by having frequent litters. Lactation, however, was practically a failure except in the case mentioned.

It was thought that the poor lactation might be due to a deficiency of vitamin B, or perhaps of a new accessory of water-soluble nature which had been mentioned by Evans (1924). With this in mind, an alcoholic extract of ether-extracted wheat embryo, made as described in Paper I, was added to the ration, which also contained the 2 per cent of wheat germ oil. This ration was fed only to the four females which had had no success with oil alone. There was considerable improvement in lactation following the addition of this extract, four litters being raised by three females which had been unsuccessful before. The improvement is shown in the following table:

	No. born	No. lived	Per cent lived
After oil.....	111	13	11.7
After oil and extract.....	41	18	44.0

The four males all received the 2 per cent wheat embryo oil and the three which lived showed no testicular degeneration.

Seven females and four males of the second generation were continued on the experiment with the 2 per cent oil addition but no extract. Two of the females showed subnormal growth at 100 days of age and the general appearance of the animals was not good, their fur appearing rough and rather yellowish. Two of the females had no litters and one had only one. One of the males was apparently infertile, which may partly account for the poor results in the females. Only two females had any success in lactation.

Egg, Ration 110. There were five females and five males on this ration. Growth was normal or above in all cases and all animals were fertile. Four of the females had repeated litters but lactation was poor. Only 10.7 per cent of the young born were weaned.

Seven second-generation females and 2 males were kept on this diet. Growth was above normal. The good fertility was mantained and lactation was improved, 37 per cent of the young surviving.

The 5 third-generation females showed decreased fertility and less successful lactation. Their

growth was above normal but the animals were very fat which possibly had something to do with their decreased fertility (Marshall, 1910). The 2 males were both fertile and made excellent growth.

Discussion of Rations in Series I

The superiority for reproduction of the egg ration (110) to the milk ration (100) cannot have been due to the slight difference in lard content. Therefore egg must contain either more vitamin E than milk contains, or there is more of some substance in it which prevents the destruction of the vitamin by oxidation.

The poor lactation on both rations was very probably due largely to a deficiency in vitamin B, especially the heat-labile fraction B (or B_1)*. This was indicated by the fact that lactation on the milk ration was so much improved by the extract of wheat embryo which is rich in that fraction. Apparently the amount of vitamin B provided by 5 per cent of yeast in these rations was not sufficient to supplement that in the dried milk and egg used.

It is difficult to make a direct comparison of these rations with the meat rations of this same series, mentioned in the previous paper (Clayton, 1930), owing to the fact that wheat germ oil and wheat germ extract additions were made to some rations and not to others. It is evident, however, that ration 110 (egg) is superior to ration 130 (liver) for reproduction, but it should be noted that ration 130 contained 15.5 per cent of lard and ration 110 only 3.5 per cent.

The fact that Daniels and Hutton (1925) secured good reproduction and lactation on a diet of milk with certain mineral additions, made it seem desirable to try the effect of feeding the milk and egg rations using the special salt mixture described in Paper I. These rations were designated as 100S and 110S.

Milk, Ration 100S. There were 10 females and 3 males on the ration. The growth of all was normal or above and reproduction and lactation were much better than on ration 100. All of the females were fertile and all had repeated litters except one which was found to have badly diseased lungs. Thirty-five per cent of the young born were weaned. The males were all fertile.

Seven females and 4 males of the second generation were kept on the ration. All of the females except one were fertile but reproduction records were not so good as those of the first generation. This was no doubt due largely to the fact that 2 of the males became sterile early in life. Three females died at the birth of their first litters. Lactation was less successful than in the first generation, 15.8 per cent of the young born surviving.

Two females and 2 males of the third generation were kept on the ration. One female had two litters and the other none. One litter of 5 was raised. One male was fertile and the other was sterile.

Egg, Ration 110S. The 7 females and 3 males placed on this ration all showed exceptionally

* The term vitamin B as used in this paper refers to the complex which includes the heat-labile factor, called vitamin B (or B_1), and the heat-stable factor vitamin G, (or B_4).

good growth. All of the females were fertile, having repeated litters. Of the young born, 31.2 per cent were weaned, which is a considerable improvement over ration 110. The males were all fertile. The 7 females and 3 males of the second generation which were kept on the ration showed growth and fertility equal to that of the first generation. Forty-four per cent of the young survived. The 4 females and 3 males of the third generation were all fertile and all but one showed very good growth. Lactation was not so successful as in the second generation, only 11.4 per cent of the young surviving.

Discussion of Rations 100S and 110S

Results from feeding these two rations show that both reproduction and lactation on the milk diet containing 15 per cent protein and 20 per cent fat (2 per cent lard) were considerably improved by using a salt mixture containing certain of the rarer minerals.

The same salt mixture used in an egg diet containing the same proportions of protein and fat improved lactation only; reproduction was already satisfactory with the unmodified Osborne and Mendel salt mixture.

Series II—High Protein, High Fat

Series II rations with protein increased to 20 per cent were as follows:

Composition of Rations
(20% protein, 20% fat, 5% ash)

Ration No.	Product (powdered)		Starch	Lard	Salts	Fe Citrate	Yeast	c.l.o.
101	Milk	75.0	15.9	—	2.0	0.1	5.0	2.0
111	Egg	45.7	43.3	—	4.0	—	5.0	2.0

Milk, Ration 101. All but one of the 10 females and 5 males on this ration showed growth which was normal or above. All of the females were fertile, but 4 died at the birth of litters. The cause appeared to be premature separation of the placenta. The fact that 3 females did not have litters until they were about 225 days old was probably because they were mated with the one male which proved to be sterile. Lactation was unsuccessful, only 9.8 per cent of the young surviving.

The growth of the 6 females and 3 males from the second generation was normal or above. All of the females were fertile at some time in their lives, but in two the first litter appeared late and in five reproduction ceased early. The early cessation of fertility in these animals was due in one case to the fact that the female had ovarian cysts and in the other four cases to the fact that the animals were mated with the only male which proved to be sterile. Lactation was much better than in the first generation animals, 48.5 per cent of the young surviving.

The 3 females and 2 males of the third generation were all fertile and lactation was almost equal to that in the second generation.

It is very apparent that by increasing the protein in the ration from 15 to 20 per cent both reproduction and lactation were improved. This ration contained no lard, as the fat content of the milk powder was sufficient to make the 20 per cent.

Egg, Ration 111. The 10 females and 6 males placed on this ration showed unusually good growth. The females had repeated litters and lactation was considerably better than on ration

110. Thirty-six and three-tenths per cent of the young born survived. All of the males were fertile except one.

The 9 females and 4 males from the second generation showed growth equal to that of the first generation. The excellent fertility was continued and lactation was greatly improved, 75 per cent of the young surviving.

The excellent growth shown in the first two generations was continued in the third. Of 4 females 3 were fertile. Lactation was less successful; only 25.4 per cent of the young were weaned. The 2 males were both fertile.

It is thus shown that by increasing the protein to 20 per cent this ration was made fairly adequate for lactation, whereas at a 15 per cent level reproduction, but not lactation, was successful Like ration 101, this ration contained no lard.

Discussion of Rations in Series II (See Table I)*

A comparison of the results of feeding the rations in this series shows that, as was the case in Series I, egg was superior to milk for reproduction. Both the egg and milk rations were superior to the meat rations of this same series (Clayton, 1930), in which round was best and liver second. It

Table I

Ration 20% protein 20% fat	No. females	Av. age birth 1st lit. days	Av. No. lit. per female to 255 days	Av. No. born per female	Per cent that lived	Remarks
101, Milk+c.l.o., no lard	10	130	1.9	14.2	10.6	4 females died at parturition. Fertile to 3rd generation.
111, Egg+c.l.o., no lard	10	102	2.7	22.3	39.4	Fertile to 3rd generation
121, Round+lard and c.l.o.	8	112	2.0	11.5	10.8	2nd generation sterile
131, Liver+lard and c.l.o	7	100	1.9	11.3	50.6	2nd generation sterile
141, Kidney+lard and c.l.o.	8	121	1.4	3.1	0.0	No second generation

should be noted, however, that neither the milk ration nor the egg ration contained any lard, while the meat rations contained about 15 per cent. As was stated in the preceding paper, the unsaturated fats in lard tend to oxidize and in the process the amount of vitamin E in the ration decreases, unless an antioxidant is present.

As regards lactation, egg was superior to milk but in comparison with the meats, liver took first place and egg second. Results on milk and round were very similar.

* For purposes of comparison, the data secured on the meat rations are included in this table.

Series III—Medium Protein, Low Fat

Composition of Rations
(15% protein, 10% fat, 5% ash)

Ration No.	Product (powdered) Skim milk	Starch	Lard	Salts	Fe Citrate	Yeast	c.l.o.
103	39.8	43.9	7.5	1.7	0.1	5.0	2.0

In this series, containing an amount of fat more nearly corresponding to that found in the human dietary, it was impossible to use either egg flake or whole milk powder, owing to their high fat content. Skim milk powder was used, however, and the results obtained with ration 103 and its modifications were among the most interesting obtained throughout the study.

Skim Milk, Ration 103. Six females and 2 males were put on the ration as originally planned. Growth was normal, but when the animals were 150 days old, only 2 females had borne one litter each and none of the young had lived. At this point 5 per cent of water was added to the ration daily, in the hope that in that way reproduction might be improved. The idea for this change in the ration came from Anderegg and Nelson (1926), who were of the opinion that the addition of water to certain highly desiccated diets improved their nutritive value for reproduction. These workers used skim milk powder diets which differed from ration 103 in that they contained no lard and the yeast and cod liver oil were added daily, instead of being mixed into a large quantity of the ration.

The addition of the water to ration 103 had no beneficial effect on the animals up to 175 days of age, when they were discarded. At autopsy scars were noted on the uterus of each, which showed that they had at some time been pregnant. The males both became sterile.

Skim Milk, Ration 103C. This ration was the same as 103 with the exception that the lard, cod liver oil, and 5 per cent of water were added daily to a basal mixture. Nine females and 3 males were put on experiment and all showed growth considerably above normal. Six of the 9 females had litters up to the time the animals were about 150 days of age. This was a remarkable improvement over the results on ration 103. Lactation, however, was a complete failure. For that reason 2 per cent of wheat germ oil was added to see if lactation would be improved. The results were very striking. Fifty-one per cent of the young born now survived. This would seem to give definite proof that more vitamin E is necessary for lactation than for reproduction. This conclusion was first made by Sure (1926), who, in working with skim milk diets, found that the requirement of the unsaponifiable matter from wheat oil was considerably greater for lactation than for preventing sterility.

Five second-generation females, and 2 males were continued on the ration with the 2 per cent oil addition. Growth continued excellent, and all of the animals were fertile. Lactation was successful in 3 of the females. Of the total young born on the ration, 49 per cent were weaned.

Discussion of Rations 103 and 103C

Results obtained on these rations seem to show that skim milk contains considerable amounts of vitamin E. The content of this vitamin in a skim milk ration seems to depend, however, on the other constituents of the food

mixture. When lard and cod liver oil are present, and the mixture is allowed to stand for any length of time, the vitamin is destroyed. If, however, the lard and cod liver oil are added at the time of feeding, together with water, considerable success in reproduction results, showing that vitamin E is present. As demonstrated by Anderegg and Nelson (1926), unsaturated animal fats, such as lard and cod liver oil, upon becoming rancid, have a destructive effect on vitamins which is associated with processes of oxidation. The wheat germ oil used in ration 103C served as an antioxidant as well as a source of vitamin E.

Series IV—High Protein, No Added Lard

The results secured on the rations of the first three series naturally led to a fourth containing 20 per cent protein and no added fat except 2 per cent of cod liver oil to supply vitamins A and D. Rations 101 and 111 of Series II really belonged to this series in that they contained no lard. A skim milk ration was included to see if by increasing the protein and omitting lard entirely, the results obtained on ration 103C might be excelled.

Composition of Rations
(20% protein, no lard, 5% ash)

Ration No.	Product (powdered)		Starch	Salts	Fe citrate	Yeast	c.l.o.
104	Skim Milk	53.0	39.2	0.7	0.1	5.0	2.0*

* Daily plus water.

Skim Milk, Ration 104. The animals on this ration got a poor start, owing to the fact that the food consumption was low. This proved to be due to the hardness of the ration. The food was soft in consistency when first mixed with water, but later became extremely dry and hard on the surface. When the animals were about 125 days old, the water was omitted from the ration and they began to improve considerably. All 6 of the females were fertile at some time but 3 died following the birth of litters. Thirty-seven per cent of the young born were weaned. Ruling out the 3 females which died, the lactation was fairly good, 58 per cent of the young surviving. Of the 3 males, 2 died but the other was fertile. Five females and 2 males from the second generation were kept on the ration for a short time. The 4 females which reached the age of 125 days had no litters. Only one male appeared to be normal.

Discussion of Ration in Series IV

Even though the second generation animals showed no reproduction, it can be said that ration 104 was much better than 103C before oil was included, in that lactation was so much better. Vitamin E was present in limited amounts, as was shown by the results in the second generation.

Ration 104 cannot be compared with others in the milk series owing to the fact that the animals got a poor start. It is difficult to compare rations 101 and 111 from Series II with the rations in Series IV owing to the fact that in these two rations the cod liver oil was mixed into large amounts of the food, while in Series IV it was added daily after the animals were about 135 days of age. A direct comparison can be made, however, of results up to the time of the daily addition of the cod liver oil to the meat rations (See Table II).

TABLE II

Ration 20% protein no lard 2% c.l.o.	No. females	Av. age birth 1st lit. days	Av. No. lit. per female to 135 days	Av. No. born per female	Per cent that lived
101, Milk	10	130	0.9	8.1	0.0
111, Egg	10	102	1.2	8.5	32.9
124, Round	8	121	1.0	6.8	3.7
134, Liver	9	117	1.2	6.4	0.0
144, Kidney	8	149	0.8	6.0	0.0

It will be noted that the order of excellence of the five protein foods for reproduction is the same in this group (Table II) as in Series II, as shown in Table I. The striking improvement in reproduction on the meat ration, following the removal of lard, and the addition of cod liver oil daily, was commented upon in the previous paper.

SERIES V—HIGH PROTEIN, TEST FOR VITAMIN B

The rations of Series V were planned in order to test for the vitamin B content of the protein foods. Milk was omitted from the series since previous work has generally shown it to contain insufficient vitamin B for normal growth and reproduction. The egg ration was as follows:

COMPOSITION OF RATION
(20% protein, no lard, no yeast, 5% ash)

Ration No.	Product (powdered)	Starch	Salts	c.l.o.
115	Egg 45.7	48.3	4.0	2.0

Egg, Ration 115. The 7 females and 3 males on this ration showed excellent growth, equally as good as on ration 111. All of the animals were fertile but their general condition was rather poor. Three had badly diseased lungs, and several were very fat. Only 4 of the females had re-

peated litters, as on rations 110 and 111, and lactation was very poor. Deficiency of vitamin B was evident. Five per cent of yeast was added late in the lives of the animals but apparently they were too old to show any response.

Four females and 3 males of the second generation were kept on the ration but growth was not so good and none of the females had litters. The males had normal testes weights but apparently the poor condition of the females prevented successful reproduction.

Discussion of Rations in Series V

The fact that the requirement for vitamin B is much greater for lactation than for growth is shown by a comparison of results on rations 111 and 115.

Ration	No. females	Av. No. lit. per female to 255 days	Av. no. born per female	Per cent that lived
111, 5% yeast	10	2.7	22.3	39.4
115, no yeast	7	3.3	17.1	6.6

Egg fed at a 20 per cent protein level apparently contains sufficient vitamin B for normal growth but not enough for successful lactation. The same may be said of liver and kidney, as shown in the previous paper. Reproduction did not seem to be affected adversely by the removal of the yeast.

Series VI—High Protein, Test for Vitamin A

The rations of Series VI were planned to test the protein foods for vitamin A content and to determine the possible beneficial effect upon reproduction of omitting cod liver oil entirely and supplying vitamin D to the animals by radiation.*

The rations were as follows:

Composition of Rations
(20% protein, no lard, no c.l.o, 5% ash)

Ration	Product (powdered)		Starch	Salts	Fe citrate	Yeast
106	Milk	75.0	17.9	2.0*	0.1	5.0
116	Egg	45.7	45.3	4.0		5.0

* Special.

The animals in this series showed unusual success in reproduction but not in lactation. In the attempt to determine the reason for this an addition of 2 per cent of wheat germ oil was first

* The animals were radiated twice a week for three minutes at a distance of 3 feet. The lamp used was a Hanovia Quartz Mercury Arc.

made to the rations at the time the animals were about 175 days of age. A little later 3 per cent more of yeast was added, making a total of 8 per cent. The results of these modifications will be taken up later in the discussion of each ration.

Milk, Ration 106. The growth of the 8 females and 3 males on this ration was normal, or above. All of the animals were fertile but lactation was successful in only one female. This animal had one litter before the oil addition and another afterward, but lost her litter following the yeast addition. Wheat germ oil cannot be said to have had any beneficial effect on lactation. After yeast one female kept some of her third litter (12 young) 28 days. The animals seemed especially subject to infections.

Two females and one male from the second generation were kept on the ration. They were all fertile but growth of the females was subnormal. One female raised a litter of 5 young following the additions of oil and yeast.

Egg, Ration 116. The 10 females and 4 males showed excellent growth, equally as good as that on ration 111. All but one of the females which were old enough had litters before the oil addition. One, however, died at parturition. Lactation was a complete failure. After the addition of oil, all the remaining females bore litters. Lactation was not improved in any case until after the addition of 3 per cent more yeast. Then one female raised a litter of 9 and two others raised 2 and one young, respectively. The males were all fertile. There was considerable lung disease on this ration.

Discussion of Rations in Series VI

As stated in the previous paper, the interpretation of results on the ration of this series is complicated by the prevalence of infections among the animals. Table III shows that by omission of cod liver oil, ration 106 was made better for reproduction than ration 101. The same cannot be said for the egg ration, however.

Table III

Ration	No. females	Av. age birth 1st lit. days	Av. No. lit. per female to 140 days	Av. size litter	Av. No. born per female	Per cent that lived
101, Milk+c.l.o.	10	130	1.00	9.0	9.0	0.0
106, Milk no c.l.o.	8	107	1.40	7.8	10.8	8.1
111, Egg+c.l.o.	10	102	1.40	7.7	10.8	32.4
116, Egg no c.l.o.	10	138	.90	6.1	5.5	0.0
134, Liver+c.l.o.	9	117	1.20	6.4	7.7	0.0
136, Liver no c.l.o.	9	114	.78	8.0	6.2	3.6
144, Kidney+c.l.o.	8	149	.87	6.9	6.0	0.0
146, Kidney no c.l.o.	8	106	1.40	9.1	12.5	8.0

Lactation was practically a failure on the rations of this series, due possibly to a deficiency of vitamin A as well as B. Table III really gives a wrong impression as to the success in lactation on ration 101 since the figures were compiled before the animals had borne very many litters. As will be seen from Table I, there was some success in lactation on this ra-

tion. However, that function was not nearly so good as it should have been and it is probable that the ration would have been improved for lactation by the addition of more yeast just as the Series VI rations were.

Table IV gives a comparison of results on all rations of this series. It will be noted that reproduction was best on the kidney ration, the milk ration being second best.

TABLE IV

Ration 20% protein no lard no c.l.o.	No. females	Av. age birth 1st lit. days	Av. No. lit. per female to 140 days	Av. size litter	Av. No. born per female	Per cent that lived
106, Milk	8	107	1.40	7.8	10.8	8.1
116, Egg	10	138	.90	6.1	5.5	0.0
136, Liver	9	114	.78	8.0	6.2	3.6
146, Kidney	8	106	1.40	9.1	12.5	8.0

SERIES VII—RAW PROTEIN, NO ADDED LARD

The final series of rations contained the protein foods in the raw state. Milk was omitted because the food value of the raw product has been studied by several workers (Mattill 1920, Daniels 1925). Raw egg, however, was included because in view of recent papers, raw egg white appears to be less easily assimilated than cooked. The ration was as follows:

COMPOSITION OF RATIONS

(15% uncooked protein, no lard, 5% ash)

Ration No.	Protein food, gms.	Starch, gms.	Salts, gms.	Yeast, gms.	c.l.o.*
117	112	54.8	4.0	5.0	2.0

* Daily.

Before being mixed with the other ingredients, the egg was always thorougly beaten with a fork.

Egg, Ration 117. The 8 females and 3 males on this ration showed normal growth up to 100 days of age, but after that some of the animals declined. Food consumption was low in spite of the fact that all seemed to have a good appetite at feeding time. The general condition of the animals was poor. In many cases the fur was very short with no lustre and there was a great deal of lung disease. One female showed a uterine infection but was fertile in spite of this fact. The other females were all fertile but reproduction records were poor owing to the fact that 2 of the males were apparently infertile, although their testes weights were not very low. Lactation was a failure. When the animals were about 191 days of age the yeast was fed separately instead of being mixed into the ration. This change was made because it was thought that the yeast was

causing too much fermentation of the food mixture as it stood in the food dishes. This change, however, was of no benefit to the animals.

Discussion of Rations in Series VII

There seems to be no explanation for the poor results on ration 117 except the possibility that there was poor assimilation of the raw egg. One of the males was showing considerable diarrhea when anesthetized which suggests the condition described by Bateman (1916) in his animals fed on a diet composed of 60 per cent of raw egg white. In spite of the poor condition of the animals, however, reproduction was not as poor on ration 117 as on ration 127 containing beef round (See Table V). Moreover, repro-

Table V

Ration 15% uncooked protein no lard 20% c.l.o. daily 50% ash	No. females	Av. age birth 1st lit. days	Av. No. lit. per female to 215 days	Av. size litter	Av. No. born per female	Per cent that lived
117, Egg	8	167	1.0	9	9.0	0.0
127, Round	8	152	0.6	10	6.5	0.0
137, Liver	8	157	1.0	7	7.0	30.
147, Kidney	9	139	1.8	6.9	12.2	32.7

duction on egg was about the same as on ration 137 containing liver. The superiority of the liver ration was shown in its ability to provide for some success in lactation. The kidney ration was much the best of the four for reproduction and a little better than the liver ration for lactation. In this series the difference in the quality of the proteins was probably one of the principal causes for the distinct variation in results, the superiority of raw kidney protein showing up rather plainly when fed at the 15 per cent level.

Summary and Conclusions

In connection with the study of the relative value of beef muscle, liver and kidney for reproduction and lactation in the rat, a similar study was also made of milk and egg and the results compared with those secured on the three meats. As in the case of the meats, the egg was fed in both cooked and raw forms. Milk, however, was fed only in dried form. The rations were planned to contain 15 and 20 per cent protein, and various supplements were used depending on the particular food constituent for which the test was being conducted. The percentage of fat was adjusted, when necessary, by the use of lard, but lard was omitted after its destructive effect on vita-

min E was noted. In the later rations, the destructive effect of cod liver oil was prevented by adding the oil daily or omitting it entirely.

The results secured from feeding rations, containing 15 and 20 per cent of protein with 20 per cent of fat, indicated that egg is superior to milk for both reproduction and lactation. When a special salt mixture containing some of the rarer minerals was used in place of the Osborne and Mendel mixture, reproduction on milk was greatly improved, indicating that the difference in results on milk and egg rations containing the Osborne and Mendel salt mixture was due partly to a difference in ash content. Lactation on both the milk and egg rations was improved by the use of the special salt mixture.

While it is impossible to make any definite statement as to the relative value of milk and egg proteins as compared to those of liver and kidney, it appears that those of dried egg and kidney are superior to the other two. Muscle protein is of lower value than that of milk, egg or the glandular meats.

When the difference in the quality of the proteins was largely ruled out by feeding them at a 20 per cent level, the vitamin E content of the five dried products was found to decrease in the following order; egg, milk, round, liver, kidney. Even skim milk contains considerable vitamin E in the absence of destructive agencies such as lard and cod liver oil. The statement of Sure (1926) that more vitamin E is needed for lactation than for reproduction was confirmed.

Success in lactation on the different rations was found to be especially dependent on their content of vitamin B(B_1). An alcoholic extract of ether-extracted wheat germ, which is rich in this vitamin, caused an improvement in lactation on both milk and beef round rations. Additional wheat germ oil and yeast also caused improvement in many cases. The superiority of dried egg over the other protein foods for lactation was no doubt partly due to its content of vitamin E. In fact, lactation on all of the rations would probably have been improved by an additional source of this vitamin.

When fed at a 20 per cent protein level and adequately supplemented with minerals and vitamins, it is probable that all of the cooked and dried protein foods tested would be adequate for both reproduction and lactation. Of the five, egg would seem to require the fewest supplements. In the raw state, kidney appears to be the most adequate. Raw egg, like raw beef round, is poorly assimilated.

The author wishes to acknowledge her indebtedness to Professor Henry A. Mattill for helpful advice throughout the work.

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DEFICIENCIES OF SYNTHETIC DIETS IN CHICK NUTRITION

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SOME years ago we reported (1) that the commonly used simplified diets are entirely inadequate for satisfactory nutrition of the chick. In many cases slow growth is the only abnormality, in others the condition suggests polyneuritis.

In an effort to obtain evidence concerning this nutritional deficiency, we weighed the internal organs (2) and analyzed the blood (3) of a number of experimental chicks reared on synthetic diets. These observations were then compared with others made on chicks suffering from a deficiency of a known vitamin, A, B, or D. Many of the experimental birds had enlarged adrenal glands, and high blood sugar, and both these abnormalities occur frequently in chicks that are on rations deficient in vitamin B. This fact strengthened our conviction that the simplified diets employed are deficient in at least one component of the vitamin B complex.

Experimental

The experimental procedure, as described previously (1), has not been changed in any essential. White Leghorn chicks are used exclusively, and all are reared under strictly laboratory conditions. The basal ration has been slightly changed, and now has the following composition:

Ration 787

Casein*	35 parts
Corn Starch	28 parts
Dried Yeast	15 parts
Lard	10 parts
Cod-liver Oil	5 parts
Cellulose	3 parts
Salt Mixture**	4 parts

* Leached with acidified water.

** Osborne-Mendel Mixture (4).

The experimental diets we have used are not described in detail, as in most cases they differ from No. 787 chiefly in the source, or amount, of the vitamin B carriers. When the percentage of this supplement was

changed, the amount of corn starch was correspondingly increased or reduced.

Our earlier attempt to identify with polyneuritis the inadequacy we were studying, had not been entirely successful. It seemed possible, however, that the vitamin B carriers employed had not been administered in large enough amounts, so it was decided to prepare the Osborne-Wakeman concentrate (5) and repeat our earlier trials. In the first series with this preparation each chick received in addition to Ration 787, 200 mgm. daily of Fraction II the first week, 400 mgm. the second, and 500 mgm. daily thereafter. However, one chick developed leg weakness, and the others did not grow more rapidly than those receiving the basal ration alone.

In the second series the chicks received Fractions II and III of the Osborne-Wakeman preparation, in the amounts given below:

Quantity of Fractions administered daily

	Fractions			*Fractions*	
Week	II	III	*Week*	I	III
1	120	100	5	700	770
2	240	260	6	700	770
3	240	260	7	1050	1100
4	440	480	8	1050	1100

As shown in Fig. 1, Group 1, all chicks grew with surprising rapidity for three weeks, but later three of the five collapsed with typical neuritic symptoms. During the attacks the leg muscles were spastic, and sometimes tremors, also mild head retraction, were noted. The three affected chicks were given skim milk forcibly, through a pipette, and two of them recovered promptly. The third regurgitated the milk, and died the following day. The chicks that recovered received skim milk *ad lib.* as long as they were under observation, and were apparently normal until the end. Two chicks developed no untoward symptoms, and one of them made truly remarkable growth. This trial was repeated, and even though the quantity of concentrate given was doubled, the result was practically the same.

The possibility presented itself that the activity of the yeast, or yeast concentrate, may have been reduced by drying or other necessary manipulation, so a comparison was made, Fig. 1, Groups II and III, of dry and of fresh yeast. A sample of starch-free baker's yeast was divided into two parts. One was dried in an air current at a low temperature, the other was kept frozen throughout the feeding trial. The ration containing dry yeast was mixed at intervals of one week or less, the other was mixed

daily, and in such a proportion that the two rations were alike except for the larger amount of water present in the fresh yeast. It was hoped that daily mixing would prevent the yeast from deteriorating, but this object may not have been accomplished. Our observations were in accord with those of Scheunert and Schieblich (6) that the drying process does not appreciably decrease the vitamin B activity of yeast. The incidence of leg weakness was practically the same in both groups, though chicks receiving dried yeast grew the more rapidly.

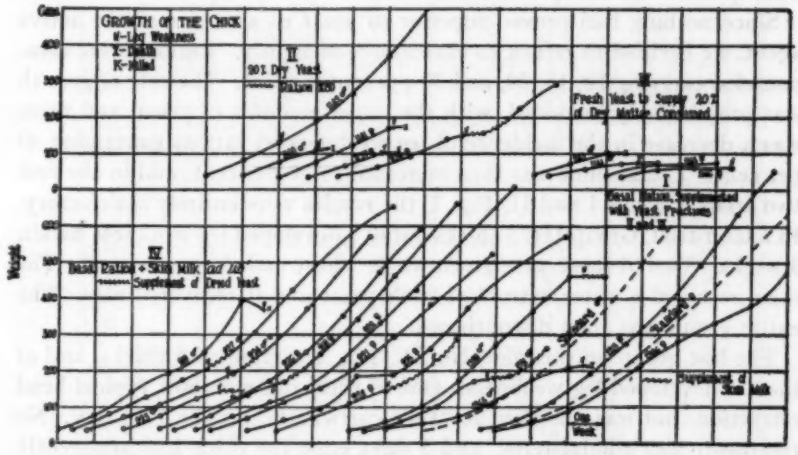

FIG. 1. The yeast concentrate and skim milk both accelerated the growth rate, but did not prevent the development of leg weakness. The dried yeast was as effective as fresh yeast.

Skim milk had brought about extremely rapid recovery in some cases, so it was decided to use it as a supplement to the basal diet, to see if it would prevent leg-weakness as well as cure it. Skim milk was offered *ad lib.*, and water withdrawn entirely. It is evident from Fig. 1, Group IV, that the rate of growth was greatly accelerated, but the incidence of leg weakness was not reduced. Of the 10 chicks shown, 6 were severely affected. Of the affected birds, 5 were treated with an emulsion of yeast and all but 1 recovered. The sixth received no treatment and was killed for blood analysis.

The disappointing experience just described led us to look elsewhere for sources of the active material. As already described (1), dried egg yolk supplements simplified diets effectively, and unpublished observations have shown that rations containing 60 per cent of wheat, or 25 per cent

of wheat middlings are also effective. Accordingly, an effort was made to prepare extracts that would be equally effective. A few of the preparations were promising, but the results were so inconsistent that this method of attack was abandoned. There remained the possibility that any unique value of egg yolk, wheat, wheat middlings, etc., in chick nutrition, may reside in the mineral constituents. Accordingly, a quantity of wheat was carefully ignited, and the resultant ash incorporated in a synthetic diet. One hundred grams of the ration contained the ash of 100 grams of wheat, but this diet was not superior to the basal diet in any respect.

Since nothing had proved superior to yeast as a carrier of the active agent, we decided to return to that source of supply. Rations were compounded carrying 10, 15, 20, and 30 per cent of yeast. The rate of growth was progressively increased, with the larger amounts of yeast, and there was a decrease in the incidence of leg weakness on rations containing 30 per cent. The amount was then increased to 40 per cent, and in the first two trials, Groups I and II, Fig. 2, the results were entirely satisfactory. In a later trial, Group III, 3 chicks out of 7 developed leg weakness within 3 weeks. Two of these were given whole wheat and they recovered. The third received a water extract of fresh yeast and it recovered also. The entire group was then discontinued.

The last group on this diet, No. IV, Fig. 2, contained 6 chicks, and of these 3 developed leg weakness. One of them did not show typical head retraction, but was observed to "turn cartwheels" on the 19th day. No treatment was administered, and 5 days later the chick had apparently recovered. Two others never exhibited acute attacks, but deformity of the legs was evident at approximately the 14th day. These also were given no treatment, and still they made unusual gains in weight.

There seemed to be no question that yeast would adequately supplement our experimental diet, if supplied in sufficient quantity, but a ration containing 40 per cent of dried yeast can hardly be classed as simplified. Another attempt was therefore made to extract the active agent, with the immediate object of obtaining the active material in soluble form, that it might be subjected to further fractionation. Accordingly, a water extract was prepared from fresh yeast, concentrated, and dried. Ten per cent of this extract was incorporated in our basal diet, and supplied to a small group of chicks with entirely satisfactory results. Later, yeast was removed entirely from the basal diet and 20 per cent of the dry extract added, Fig. 3, Group I, with results that were almost entirely satisfactory.

Group II was started primarily to study the potency of the extract prepared from baker's yeast. For the first week their ration contained

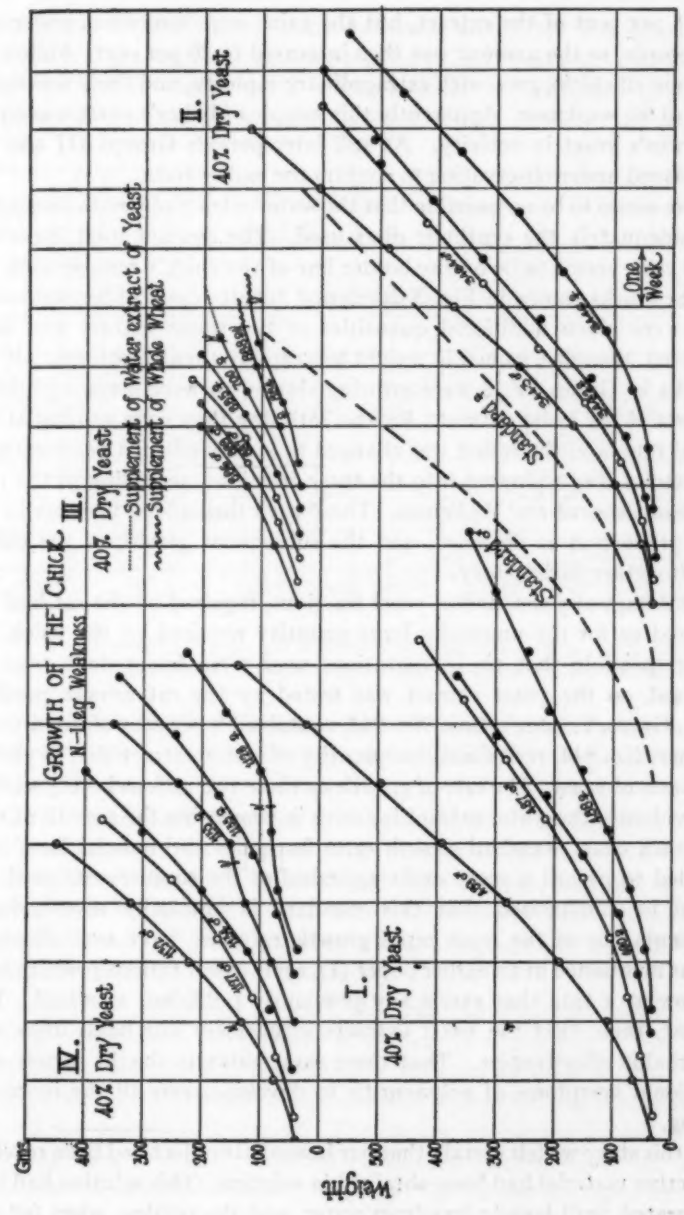

FIG. 2. At least 40 per cent of dry yeast must be included in simplified diets in order to make them adequate for chick nutrition.

only 15 per cent of the extract, but the gains were somewhat under our best records, so the amount was then increased to 20 per cent. Following that time all chicks grew with extraordinary rapidity, and there was never a hint of leg weakness. Apparently this sample of baker's yeast was equal to brewer's yeast in activity. At still later periods Groups III and IV were placed under observation to confirm the earlier tests.

There seems to be no question that the water extract of yeast can supplement adequately the synthetic diets used. The amount used, however, 20 per cent, seems to be on the border line of the chick's requirement, for 2 of the chicks shown in Fig. 3 developed definite cases of leg weakness. They were given additional quantities of the water extract and both recovered, though 1 gained in weight less rapidly than the others. Of the 4 chicks in Group IV, 2 were growing slowly, so were given additional amounts of the yeast extract. By the 25th day they were gaining at the desired rate, and the ration was changed to a reconstituted yeast extract. The extract was separated into the three fractions, according to the procedure of Osborne and Wakeman. These were then added together in the same proportion as obtained, and the subsequent growth of the chicks was altogether satisfactory.

The dosage of yeast, and of yeast fractions, required by the rat had not prepared us for the unusually large quantity required by the chick. It seemed possible that the preparations used were less potent than we supposed, so the yeast extract was tested by the rat growth method, Fig. 3, Group V. One ration, No. 943, contained 9 per cent of dried yeast. Another, No. 944, contained the quantity of water extract that is yielded by 9 parts of yeast. The rate of growth on these two rations is very similar, and evidently the water extract is active in promoting the growth of rats.

In each chart standard growth rates for males and females have been included to permit a more exact appraisal of the supplements used. It should be emphasized that this standard is unusually severe, for it represents one of the most rapid growth rates we have ever observed. As was mentioned in an earlier paper (7), some of our rations permit chicks to grow at a rate that excels any previously published standard. It is evident, then, that the yeast extracts supplement our basal diets with remarkable effectiveness. That these same extracts should permit even occasional symptoms of polyneuritis to develop seems all the more surprising.

At this stage we felt certain that our immediate object had been reached; the active material had been obtained in solution. This solution had been evaporated until largely free from water, and the residue, when fed at a

level of 20 per cent or higher, made the ration adequate. It seemed certain, however, that most of this dry matter is inert material, and that a suitable procedure would yield a much more concentrated product. This phase of the problem has not been prosecuted actively, but we did

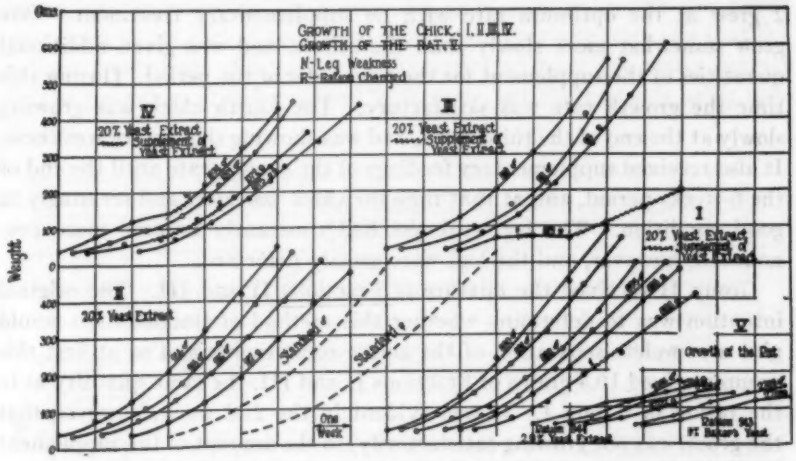

FIG. 3. The active agent in yeast can be obtained in water soluble form. Large amounts are required by the chick, however, if the extracts are used to supplement simplified diets.

attempt to decide whether the active material could be obtained in more concentrated form by fractionating the water extract by the procedure of Osborne and Wakeman.

An extract of brewer's yeast was therefore separated into the three fractions, then recombined in the same proportion, and fed at a level of 20 per cent. Two of the 4 chicks developed leg weakness, and in one the symptoms were acute, with "cartwheels" and marked head retraction. After separate, daily administration of additional quantities of the mixed fractions, the chicks recovered completely.

This experience convinced us that the Osborne-Wakeman procedure does not greatly reduce the potency of the active agent, and it was decided to make a beginning at studying the fractions separately. Fraction I was prepared by the method of Osborne and Wakeman, and evaporated to dryness in a current of warm air. The filtrate, containing Fractions II and III together, was not subjected to further fractionation, but the mixture was evaporated *in vacuo* to a syrup. On further drying in an air current it was reduced to a gum, containing 27 per cent of water. This

material was stored in the ice box. The amounts fed, as described later, are calculated to a water free basis.

A portion of the fractions was recombined, Group I, Fig. 4, and fed at a level of 20 per cent. The ration contained in each 100 grams, 9.6 grams of Fraction I and 10.4 grams of Fractions II and III. Of the 4 chicks, 2 grew at the optimum rate with no supplementary treatment. One grew somewhat more slowly than the others, and was given additional quantities of the supplement for the remainder of the period. During this time the growth rate was satisfactory. The fourth chick was growing slowly at the end of the third week, and was showing signs of leg weakness. It also received supplementary feedings of the concentrate until the end of the 6-weeks period, and at that time the chick was alert and seemingly in good condition. The leg weakness had constantly become more pronounced, however, and the legs were grossly deformed.

Group II received the mixture of Fractions II and III. The original intention was to determine whether this method of fractionation would give a complete separation of the active constituents and so at first this group received 10.4 grams of Fractions II and III, the same quantity as in the ration of Group I. It was evident in the 2nd week, however, that the group was not growing satisfactorily, so the amount of the supplement in the ration was doubled on the 12th day. One of the group gave some evidence of leg weakness the same day the ration was changed. The vitamin supplement was administered in increased amounts for a brief period, and then the chick had completely recovered. Another chick developed an acute type of leg weakness on the 40th day. It received practically the same treatment, became normal in appearance, and gained significantly in weight.

Group III received for 12 days a ration containing 20 per cent of Fraction I, but was obviously a failure. All chicks were growing poorly, and two gave definite evidence of leg weakness at that time. The percentage of Fraction I was then increased to 40 per cent, and though another chick gave evidence of leg weakness on the 14th day, all began to gain more rapidly. The affected chicks all recovered in a few days without treatment, but the fourth chick of the group had a definite case of leg weakness on the 39th day, though there were no acute symptoms.

It is seen, then, that the mixture of fractions received by Group I was fairly satisfactory. This ration contained 10.4 per cent of Fractions II and III, but evidently the favorable response of the chicks can not be credited entirely to this portion of the extract. As has just been mentioned, Group II responded poorly when they received this material, at the same

level, as the sole source of vitamin B. Even when this adjuvant was doubled, the chicks did not do better than those in Group I. Evidently the ration of Group I owed part of its effectiveness to the inclusion of Fraction I. This fraction, however, is unsatisfactory as the sole source of the water soluble vitamins, for Group III grew slowly though the ration contained 40 per cent of that preparation. We conclude from this that the active constituents can not be easily separated from the water extract by the procedure of Osborne and Wakeman, but this phase is being continued.

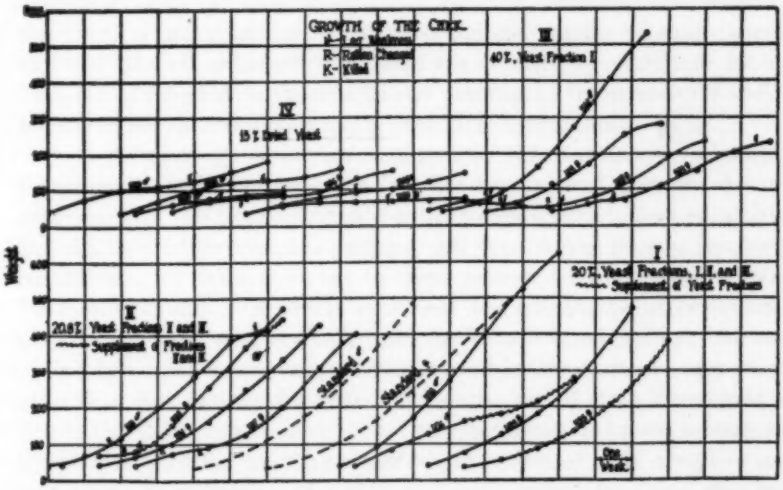

FIG. 4. Fractionation of a water extract of yeast by alcohol was not helpful in increasing the concentration of the active material.

Group IV received our basal ration and is included merely to show that the conventional simplified diets are inadequate for the chick. All members of this group grew poorly, and all developed leg weakness.

SUMMARY

1. Simplified rations must be fortified with approximately 40 per cent of dried yeast if they are to be adequate for the chick.

2. The active constituents of fresh yeast are readily obtained in water soluble form.

3. On a dry basis, 20 per cent of the water extract is roughly equivalent to 40 per cent of dried yeast.

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THE NUTRITIONAL POTENCY OF FRESH, COOKED, DRY AND ALCOHOL-EXTRACTED LIVER*†

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FROM the large number of reports in the literature concerning the nutritional potency of calf and codfish livers, the lay reader may be led to infer that such nutritional properties are specific for these two species of animals alone. In more recent years, however, investigations show that the livers of other species of fish than the cod possess vitamin potency and that the oil obtained from them can be used as substitute for cod liver oil. In regard to the nutritional potency of the livers of domestic animals other than the calf there is very little information.

It is the purpose of an investigation at the Kentucky Agricultural Experiment Station to ascertain whether the livers of domestic animals other than the calf have nutritional potency comparable to that of calf liver and can be used for the same purpose, calf liver having already become so expensive as to prohibit its use by some persons who may need it most. Furthermore, raw liver unless kept near the freezing point deteriorates very rapidly and soon becomes unfit for human consumption. Because of this fact the livers of animals slaughtered on the farm are only partly utilized or lost entirely for nutritional purposes. If fresh liver could be desiccated and preserved without its nutritional potency undergoing serious deterioration, such a process would permit of more complete conservation and utilization of a valuable food product for those who may need it most. Accordingly, through the cooperation of the Department of Animal Husbandry and the Division of Veterinary Science at the Kentucky Agricultural Experiment Station, livers from the following animals, calf, steer, lamb, pig, hog, colt, horse, chicken, rat and codfish were obtained for chemical analysis. The fresh livers were cut in small pieces, placed in porcelain dishes and heated with an occasional stirring at approximately 100°C, in an electric oven until free from moisture, which required from 24 to 48 hours. The moisture-free liver was then ground in a Wiley mill and placed in airtight glass containers. Tests for the presence of catalases, peroxidases and oxidases on portions of the

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

† This paper was presented before the Division of Biological Chemistry at the 78th meeting of the American Chemical Society held at Minneapolis, Minn., Sept. 9 to 13, 1929.

moisture-free liver with a fresh alcoholic solution of gum guaiacum and hydrogen peroxide showed that these enzymes had been destroyed in the process of drying.

The results of the chemical analyses of the material prepared as described above, are given in Table I.

After the analyses had been made, the following experiments were planned for the purpose of ascertaining whether the livers that contained the larger amounts of mineral matter, fat and nitrogen possessed greater potency than those low in these constituents.

Forty white rats, having an average weight of approximately fifty grams, were placed in ten glass cages, four rats to each cage. The cages were like those shown in photograph No. 1 and were constructed so as to eliminate any possible source of contamination from metals, a condition which is not possible with the ordinary type of cage made from galvanized iron wire such as is generally used in nutritional experiments with small animals.

In the photograph, the cage on the right is in an upright position with the glass plate which forms the top in place. The cage on the left is turned on its side, with the top removed, to show the construction more clearly.

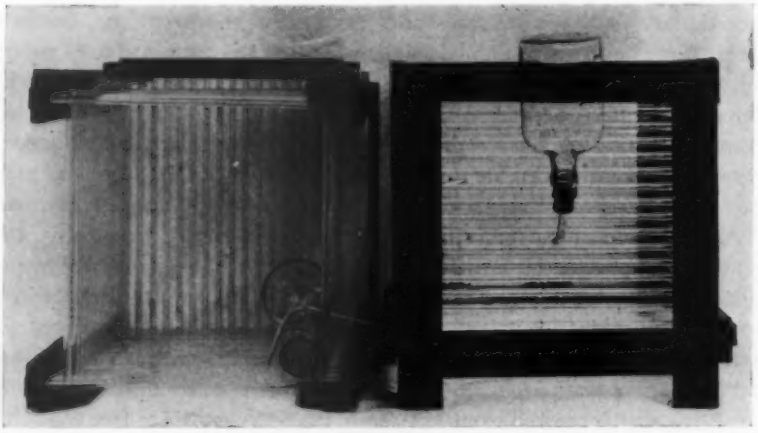


FIG. 1

The walls and bottom of the cages are made from 14-inch sections of heavy-wall glass tubing having an outside diameter of from 7 to 8 mm. The cage is built up in the form of a pen having inside dimensions of approximately 12″×12″×12″. The ends of the tubes that form the walls

TABLE I

THE MINERAL AND NITROGEN CONTENT OF THE MOISTURE-FREE NORMAL LIVERS OF SOME DOMESTIC ANIMALS. PER CENT.

Sample Nos.	1 Calf	2 Lamb	3 Steer	4 Hog	5 Pig	6 Horse	7 Colt	8 Chicken	9 Rat	10 Cod Liver chum
Copper (Cu)	.013	.028	.018	.0015	.0009	.002	.040	.0016	.0015	.0018
Iron (Fe)	.036	.039	.020	.073	.097	.138	.114	.046	.042	.166
Manganese (Mn)	.0003	.0007	.0004	.0004	.0018	.0004	.0005	.0007	.001	.001
Zinc (Zn)	.040	.031	.042	.033	.0213	.050	—	.034	.020	.023
Calcium (Ca)	.028	.033	.026	.023	.046	.023	.047	.054	.024	.122
Magnesium (Mg)	.161	.075	.217	.091	.113	.067	.103	.092	.100	.072
Phosphorus (P)	1.122	1.21	1.105	.958	1.22	.555	.910	.791	1.360	.769
Potassium (K)	.896	.872	.796	.861	1.160	.868	—	.627	1.522	.320
Sodium (Na)	.772	.625	.809	.766	.354	.572	—	.392	.965	.550
Sulfur (S)	.584	.464	.664	.297	.736	.416	—	.387	.616	.517
Nitrogen (N)	10.24	11.18	10.52	7.74	10.72	10.65	—	7.10	9.42	7.34
Protein (Nx6.25)	64.00	69.89	65.75	48.37	67.00	66.56	—	44.38	58.87	45.90
Fat	10.41	13.36	—	7.52	12.73	10.24	10.41	48.95	12.33	35.32

1 Composite sample from two calves.
2. Average of three independent analyses: (a) one liver, (b) composite of 3 livers, and (c) a composite of 4 livers; all from lambs about 4 months old.
3. From a steer about 2 years old.
4. From a hog about one year old.
5. Composite sample of 4 livers from pigs about 3 months old.
6. Average of two independent analyses of the livers of two horses about 8 years old.
7. Average of two independent analyses of the livers of two colts that died soon after birth.
8. Composite sample of the livers of 8 hens, about two years old.
9. Composite sample of the livers of 40 albino rats one year old.
10. The commercial product after removal of most of the oil.

extend about one inch after crossing at the four corners into vertical grooves made at the proper points in four angle wood posts. The wood posts are fastened to each other with cross strips. The ends of the glass tubes that form the bottom of the cage extend beyond the walls of the cage and fit into a horizontal row of holes made in two cross pieces in the same plane on opposite sides of the cage. The tubes that form the bottom of the cage are placed about 8 mm. apart to allow the excreta to fall through into trays beneath the cages. A glass plate 12″×12″ rests on the top row of the walls of the cage and forms the top of the cage. Food is placed in a glass tube container which is supported in a vertical position and held in place by bent glass tubing in one corner of the cage. The lower end of the feeding tube has lips bent in its rim through which the rats procure their food, *ad libitum*. The lower end of the tube rests in a glass dish which supports the column of food and tends to prevent the rats from wasting it. Distilled water condensed in a quartz tube is available all the time from a glass water bottle attached to the wood frame that surrounds the cage.

After marking and determining the weight of each rat they were placed in the cages and fed a synthetic ration having the following composition:

Starch	50%
Casein	20 "
Cane sugar	12 "
Lard	6 "
Filter paper	10 "
Mineral salt mixture	2 "
	100%

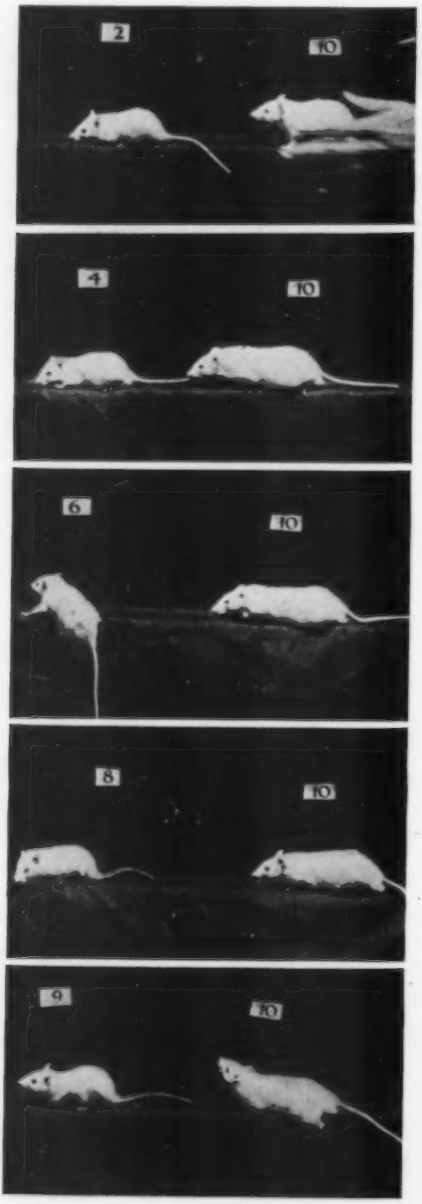
The mineral salt mixture contained the following compounds: Sodium chloride, magnesium sulfate, postassium sulfate, potassium dihydrogen phosphate, monocalcium phosphate and ferric citrate. The components of the synthetic diet were thoroughly mixed by hand in a large porcelain dish with enough water to form the consistency of dough and then dried in an electric oven at approximately 100°C until free of moisture, after which the mass was ground fine in a Wiley mill using a specially constructed, hard, brightly polished steel sieve instead of the soft, brass sieve with which the mill is ordinarily equipped.

The rats were fed the synthetic ration for seventeen days during which time they lost weight at the rate of about 1 gram per rat each day and acquired an emaciated condition. On the eighteenth day the rats in

TABLE II

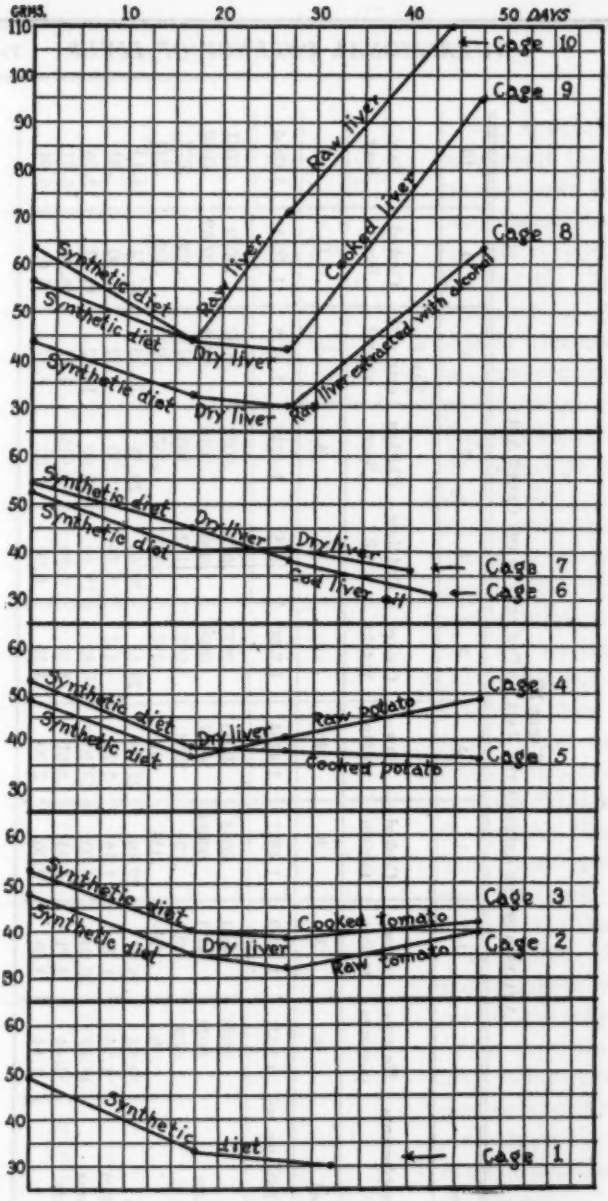
AVERAGE WEIGHTS OF RATS RECEIVING A SYNTHETIC DIET WITH VARIOUS LIVER AND VEGETABLE SUPPLEMENTS

Cage Nos.	1	2	3	4	5	6	7	8	9	10
	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
Av. wt. of 4 rats at beginning of experiment. 5/29/29	49	48	53	49	52	54	53	44	64	57
Av. wt. of rats after feeding 17 days on a synthetic diet. 6/15/29	33	35	40	37	38	45	39	32	44	44
Av. loss in wt.	16	13	13	12	14	9	14	12	20	13
Synthetic diet supplemented with dry livers 4 gms. per rat per day. 6/15/29	No supplement	Beef	Hog	Pig	Horse	Lamb	Chicken	Calf	Colt	Raw beef 16 gms. per day
Wts. after feeding 10 days, 8 dry and 1 raw liver supplement. 6/25/29	31	32	38	41	38	39	40	30	42	72
Additional supplements	None	Raw tomato	Cooked tomato	Raw Irish potato	Cooked Irish potato	Cod liver oil 2 cc. each per day.	No other supplements.	Raw liver extracted with alcohol	Cooked liver	Raw liver
Wt. of rats at conclusion of experiment	All dead 6/29/29 30	3 rats 40	3 rats 41	4 rats 49	3 rats 36	2 rats 31	4 rats 36	3 rats 64	4 rats 96	4 rats 118
Gain+; Loss−	−1	+8	+3	+8	−2	−8	−4	+34	+54	+46
Date chloroformed	—	7/17/29	7/17/29	7/17/29	7/17/29	7/11/29	7/1/29	7/17/29	7/17/29	7/17/29



eight of the cages were fed finely ground, moisture-free liver of the calf, lamb, steer, hog, horse, colt and chicken, respectively, for ten days. Each rat received approximately 4 grams of the dry liver per day which was the equivalent of 16 grams of fresh liver. In one cage the rats were given no supplement, and in another they were given raw beef liver, for comparison. It was expected that by this method of feeding the liver having the greatest nutritional potency would produce the most rapid and largest gains in the weights of the emaciated rats. However, after feeding the dried livers as supplements to the synthetic ration for ten days and weighing each of the rats daily, it was found that none of the rats had made any appreciable gains, neither did they make any gains during the second period in which the amount of dry liver in their food was doubled.

Taking the average weights of the rats after they were fed the synthetic ration for seventeen days, and the average weights after they were fed the dried livers of the different species of animals as supplements for ten days, and the final weights after receiving additional raw and cooked liver and vegetable supplement, as indicated in Table II, the following graphs were plotted.



Cage 1. Synthetic diet, no supplements. Cage 2. Synthetic diet, +dry beef liver, 10 days, +raw tomato 21 days. Cage 3. Synthetic diet, +dry hog liver, 10 days, +cooked tomato 21 days. Cage 4. Synthetic diet, +dry pig liver, 10 days, +raw potato, 21 days. Cage 5. Synthetic diet, +dry horse liver, 10 days, +boiled potato 21 days. Cage 6. Synthetic diet, +dry sheep liver, 10 days, +cod liver oil 27 days. Cage 7. Synthetic diet, +dry chicken liver, 16 days. Cage 8. Synthetic diet, +dry calf liver, 10 days, +alcohol-extracted beef liver, 21 days. Cage 9. Synthetic diet, +dry colt liver, 10 days, +cooked beef liver 21 days. Cage 10. Synthetic diet, +raw beef liver, 31 days.

TABLE III
ANALYSES OF THE ASHES OF THE RATS, AS PER CENT OF THE CRUDE ASH

Cages	1	2	3	4	5	6	7	8	9	10
Ration and Supplements	No supplements	Raw tomato	Cooked tomato	Raw potato	Cooked potato	Cod liver oil	Chicken liver	Raw liver extr. with alcohol	Cooked liver	Raw liver
No. of rats ashed	4	3	3	4	4	2	4	3	3	3
Average gms. of ash per rat	0.8321	1.2369	1.4332	1.4264	1.2390	1.3151	1.4245	1.5428	1.7885	3.0664
Copper	.0053	.0063	.0042	.0043	.0057	.0053	.0052	.0082	.0049	.0061
Manganese	.0022	.0013	.00084	.0007	.0008	.0063	.0016	.0019	.00089	.0010
Zinc	.069	.090	.091	.063	.095	.152	.183	.071	.106	.077
Iron	.39	.390	.336	.31	.297	.422	.332	.403	.298	.232
Calcium	29.98	28.47	29.16	27.68	29.83	30.46	29.35	25.33	27.73	15.13
Magnesium	1.25	1.42	1.16	1.24	.872	1.264	1.30	1.32	1.30	.88
Phosphorus	18.63	20.04	19.00	18.10	18.82	18.50	18.66	19.82	19.32	13.56
Potassium	2.44	3.91	3.54	3.61	3.50	4.89	.946	4.72	8.31	8.51
Sodium	1.84	3.08	2.30	2.00	3.10	6.00	1.02	3.57	6.05	6.94

TABLE IV

ANALYSES OF THE MOISTURE-FREE RAT FECES. PER CENT

Cage Nos.	1	2	3	4	5	6	7	8	9	10
Ration and Supplements	No supplements	Raw tomato	Cooked tomato	Raw potato	Cooked potato	Cod liver oil	Chicken liver	Raw liver extr. with alcohol	Cooked liver	Raw liver
Ash	5.83	6.49	7.83	5.42	6.14	4.77	4.86	5.69	4.56	6.34
Copper	.0029	.0039	.0036	.0031	.0037	.0093	.0039	.0202	.0162	.0098
Manganese	.0042	.0036	.0038	.0025	.0051	.0023	.0028	.0042	.0061	.0060
Zinc	.0204	.0576	.0543	.0449	.0407	.0475	.0685	.0482	.0865	.0740
Iron	.724	.557	.775	.268	.543	.684	.652	.583	.611	.771
Calcium	.422	.656	.706	.127	.488	.388	.304	.516	.255	.115
Magnesium	—	.130	.419	.138	.371	.125	.189	.161	.216	.144
Phosphorus	.713	1.19	1.21	.720	.858	.893	.944	.996	.913	1.17
Sodium	.387	.528	.448	.356	.426	.724	.324	.371	.496	.631
Potassium	.550	1.39	1.43	1.53	1.05	1.35	.677	.881	.477	.890
Nitrogen	4.72	3.40	3.34	3.62	5.78	5.40	8.16	6.29	5.82	5.50

Near the close of the experiment the photographs shown on p. 54 were made of the surviving rats in cages 2, 4, 6, 8, 9 and 10. These are presented for the purpose of comparing the rats receiving the different supplements. The numbers above the rats are the cage numbers and the diet and supplements given to the rats in each of the cages are given in the legend accompanying the graphs. At the conclusion of the experiment, the rats surviving were chloroformed, the intestinal tracts removed and the carcases dried at 100°C for analysis. Those that had died previously were prepared for analysis in the same way.

Table No. III gives the results of the analyses of the ashes of the rats in each experiment. The results of the analyses were calculated as percentages of the ash to afford a uniform basis of comparison.

After the rats in all the cages had been fed the synthetic diet for 17 days, the feces from

each of the cages were collected, dried at 100°C and analyzed at the conclusion of the experiment to ascertain if any marked variations in their mineral and nitrogen content occurred as a result of the feeding of the different supplements (Table IV).

Discussion of the Results

There are a few points of interest in connection with the results obtained in these experiments which are worthy of brief discussion.

In the analyses of the livers of the different species of animals, the copper content of the calf, lamb and steer liver was approximately ten times more than the amount found in the liver of the hog, pig, horse, chicken, rat and in cod liver chum. The results for iron and manganese are slightly higher in the livers that contained the smallest amounts of copper. The iron content of the livers of the colt, horse, pig and hog was approximately three times as much as that found in the livers of the other domestic animals analyzed. The results for zinc, calcium, magnesium, potassium, sodium and sulfur do not show any wide variations that might be regarded as having particular significance. The livers having the largest copper content also have the largest phosphorus and nitrogen content; however, the pig liver is low in copper but contains as much phosphorus and nearly as much nitrogen as any of the other livers. The chicken liver contained about four times as much fat as any one of the other livers. It therefore appears that the livers of the calf, lamb, steer and pig are about equally well supplied from the standpoint of minerals and nitrogen and therefore might be expected to have similar nutritional potency insofar as it can be ascertained from chemical analysis. However, calf liver has attained a much wider reputation for its nutritional potency than either lamb or pig liver.

The chief point of interest, and therefore of greater significance than any of the other results obtained in connection with these experiments, is the fact that livers having nutritional and vitamin potency lose these very desirable properties upon heating and drying at 100°C in air. Table II shows that the rats receiving thoroughly desiccated liver of calf, lamb, steer, hog, pig, colt, horse and chicken, respectively, as supplements, failed to produce any substantial gains in weight during a period of ten days' feeding, in comparison with rats fed an equivalent amount of raw beef liver during the same time. The results with dry livers show that the nutritional and vitamin factors associated with these livers are thermolabile and probably catalytic, oxidative and enzymatic in their nature.

The experiments further indicate that when raw liver is digested and extracted with 95 per cent alcohol, and dried in air by means of an electric fan, a considerable portion of its nutritional potency has been lost, as is to be observed in the table of weights of cage No. 8 and curve No. 8 and comparing rat No. 8 with rats No. 9 and No. 10 in the photographs. The results obtained from feeding as supplements raw tomatoes and raw Irish potatoes in comparison with these vegetables cooked, indicate that the enzymes contained in the raw food materials are important factors in their nutritional potencies. The effect of the raw and dry liver, and of the raw and cooked vegetables when fed as supplements to an inadequate synthetic food is shown to a better advantage in the curves plotted from the weights of the rats and the photographs made of some of the rats during the experiment.

With one or two exceptions the ash analyses show rather uniform results for most of the constituents of the ashes of the different lots of rats. In cage No. 1 the check rats contained less than 1 gram of ash per rat whereas in cage 10 the rats averaged a little more than 3 grams of ash per rat. There is a very marked diminution in the calcium and phosphorus content of these rats in comparison with the calcium and phosphorus content of the rats receiving the other supplements. At the present time no satisfactory explanation is apparent for this rather wide variation in the calcium and phosphorus of the ash of the rats in this experiment and further experiments in this connection are contemplated.

The analyses of the feces of the rats during the time they received the various supplements afford some points of interest. The feces of the rats fed the synthetic diet only, contained more crude ash than those of the rats which received raw potato, cod liver oil, chicken liver, alcohol-extracted liver and cooked liver, after a period of feeding on dried liver. The percentage of copper was approximately three times as much in the feces of the rats that received cod liver oil, alcohol-extracted liver, cooked liver and raw liver as supplements. The results for manganese and zinc are also higher in the feces of the rats receiving the same supplements. The iron, calcium, magnesium and phosphorus content of the feces of the rats fed raw potato as a supplement are less than they are in most of the feces of the other rats in the experiment. The feces of the rats fed cooked potato contained considerably more nitrogen than those fed raw tomatoes cooked tomatoes and raw potato supplements. The rats fed chicken liver, alcohol-extracted liver, cooked liver and raw liver, respectively, contained considerably more nitrogen than any of the other feces except those from the rats receiving cooked potato. Apparently a part of the

liver supplements was not completely digested and assimilated and therefore the feces contained a higher percentage of nitrogen.

SUMMARY

Rats fed on a synthetic diet for 17 days declined rapidly during this time and failed to make any appreciable gains in weight when fed, as supplements, moisture-free livers that had been dried at 100° C in air, whereas rats receiving equivalent amounts of raw liver, in connection with the synthetic ration, made rapid gains. Other rats receiving raw potatoes and tomatoes as supplements to the synthetic diet and dry liver, made appreciable gains, whereas rats fed equivalent amounts of cooked potatoes and tomatoes made little or no gains in weight.

THE EFFECTS OF HIGH SUGAR DIETS ON THE GROWTH AND STRUCTURE OF THE RAT

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THE consumption of sugar in the United States has steadily increased from 38 pounds *per capita* in 1875–1879 to between 110 and 115 pounds in 1924–1927 (Commerce Yearbook, 1929). Even in 1901, von Bunge (1901) pointed out certain dangers to human health from the increasing consumption of sugar. The belief that excessive use of sugar is unwholesome appears widespread among both the medical profession and the laity. The alleged injurious effects are both direct and indirect. As a direct effect, dental caries is said to occur through acid products of sugar fermentation (Harris, 1928), although this has been disputed (Friedenwald and Rurāh, 1905). Furthermore it is asserted that sugar in concentrated form causes gastric irritation (Brandl, 1918, Sherman, 1928), produced by the dehydration (Rose, 1927, McLester, 1927), or through toxic products of fermentation (Friedenwald and Rurāh, 1905, McLester, 1927, Harris, 1928), or through interference with digestion (Schule, 1918, Miller *et al*, 1920). However, no such injurious effects were noted by Snyder (1904), with human diet containing about 10 per cent of sucrose; by Carlson, Hektoen and Le Count (1916), in rats with diet including only about 2 per cent of sucrose or glucose; or by Cori (1925), who fed rats with glucose in concentrations up to 80 per cent. Excessive sucrose may also be absorbed without inversion, in which case it is excreted as a foreign body by the kidney (Fitch, 1918, McLester, 1927, Sherman, 1928).

Aside from these possible direct effects, a large consumption of sugar may be indirectly detrimental, especially to children, through loss of appetite, with resultant underfeeding (McLester, 1927, Harris, 1928). Or the appetite may become perverted, resulting in an unbalanced diet and deficiency disorders. Such disorders may be due to an inadequate intake of protein, fats or salts, especially of calcium and iron (von Bunge, 1901, Harris, 1928), or of vitamins (Harris, 1928). This point of view has been emphasized recently by the Committee on Nutritional Problems of the American Public Health Association (Sherman *et al.*).

Within the past year the question has been raised from another standpoint. On account of the chemical purity of commercial sucrose, it has

recently been used in nutritional experiments in relatively large amounts, up to 80 per cent of the diet (Evans and Burr, 1928, Burr and Burr, 1929, McAmis, Anderson and Mendel, 1929). In view of the prevailing opinion concerning the various possible injurious effects of such diets it was thought desirable to make a careful experimental study of the question, using rats fed high sugar diets from weaning time up to the adult stage. I am greatly indebted to my colleague, Professor George O. Burr, for advice and assistance in the preparation of the diets used.

Material and Methods

The animals used in the present experiments were from six litters, 36 rats in all. They were from the Long-Evans strain of pied (hybrid) rats, reared in the Institute of Anatomy. At the weaning age of 21 days, the rats were divided into three groups: (a) controls on 45 per cent starch diet, 6 males and 9 females; (b) test rats on 45 per cent sugar diet, 6 males and 9 females; (c) test rats on 80 per cent sugar diet, 6 females. In the first two groups, the litters were divided so as to provide litter-mate controls of the same sex and similar weight. In the third group, the 6 females were from a single litter.

The diets used were as follows, parts by weight being indicated. The accessories were fed separately to each rat daily so as to insure the necessary vitamin intake. The amount of yeast and wheat germ was trebled during lactation.

Diet No. 162. 45 per cent starch (control).

Corn starch (cooked)	45
Commercial casein	30
Lard	20
Salts (McCollum's mixture 185)	4.5

Daily accessories; 3 drops of cod liver oil, 0.3 gram dried yeast (Northwestern), and 0.3 gram wheat germ.

Diet. No. 561. 45 per cent sugar.

Commercial sucrose	45
Commercial casein	30
Lard	20
Salts (McCollum's mixture 185)	4.5

Daily accessories; same as in diet 162.

Diet No. 551A. 80 per cent sugar.

Commercial sucrose	80
Commercial casein	16
Salts (McCollum's mixture 185)	4

Daily accessories; same as in diet 162, with addition of 10 drops of lard daily.

The rats were kept in roomy wire cages, in which tap water was supplied from bottles with glass tube outlets. Body weights were taken weekly in the earlier part of the experimental period, and at intervals of 2 or 3 weeks later. In calculating the average body weights for the curves in Fig. 1, the weights of the females during pregnancy were omitted.

The length of the experimental periods varied somewhat in the different groups, as shown by the ages of autopsy (Table I). The aim was to continue the experiment until practically adult weight was reached. The youngest group (females on 80 per cent sugar diet) was killed at average age of 148 days, while the oldest group (females on 45 per cent sugar diet) averaged 200 days at autopsy.

In the 80 per cent sugar-fed female rats, the time of the vaginal opening was noted, and the successive estrus cycles were observed by daily vaginal smears with the technique of Evans. These 6 females were all bred to normal stock males, and had 1 litter each. Three females on the 45 per cent starch diet and 4 on the 45 per cent sugar diet were similarly bred, and bore 2 litters each in all but 2 cases. Where possible, weights were taken of the newborn (usually including ingested milk). Weights were also taken of the litters at the weaning age of 21 days, to test the adequacy of lactation on the various diets.

At autopsy the following technique was used, the results being recorded on printed cards. The body weight was taken and (in the females) a vaginal smear to determine the final stage of the estrus cycle. Then the rat was killed by chloroform. The external appearance of the body was carefully noted. The nose-anus length was measured with calipers, the body being extended by slight traction. The tail length was measured with the body suspended from the tip of the tail. The head was then removed, the plane passing just behind the cranium and the hyoid bone. While the head was being weighed and dissected, the body was suspended by the tail to obtain uniform drainage of blood from the viscera. The submaxillary glands (included with the head) and the eyeballs were next taken. The external orbital glands must not be mistaken for parotid. After the cranial roof had been removed with bone forceps, the brain was exposed and separated by clipping the cranial nerves. Next the hypophysis was taken. Through the thin roof of the tympanic cavity, the presence or absence of pus was noted, this being verified by removing the roof. In the last group of rats a midsagittal section of the head was made with a heavy cartilage knife, and the condition of the nasal fossae was

recorded. The teeth were also examined. In the first two groups, the skulls were preserved intact and were cleaned by boiling in 1 per cent soap powder solution in order to examine the teeth more carefully. In general, all dissected parts were preserved (until weighing) in covered glass dishes containing moist filter paper, to avoid drying.

The peritoneal cavity was next opened, and the condition of the various abdominal viscera was observed before dissection. Then the reproductive tract (testes, epididymides; or ovaries, uterus) and spleen were taken. Next the entire gastro-intestinal tract, including contents, with mesentery and pancreas was removed and weighed together. Then the mesentery was separated, and the wall of the stomach and intestine was slit open throughout to permit inspection of the contents and the mucosa. Pancreas, and empty stomach and intestines were then separated. The suprarenal glands, kidneys and liver were next taken.

The thorax was next dissected, noting the appearance of the skeleton. Thymus, heart (opened to remove blood clots) and lungs were removed in order. The larynx, with adjacent trachea, pharynx and thyroid gland, was usually preserved intact for histological study, but in a few cases the thyroid glands were removed and weighed separately. The integument was next separated, and the *tela subcutanea* removed from it as completely as possible. In the first two groups of rats, the spinal cord was next removed. The skeleton and musculature were then weighed together, after removing the extraneous fat, vessels, etc. In the last group of 6 rats, the spinal cord was not removed, but its estimated weight was deducted to obtain the weight of skeleton and musculature.

The head, gastro-intestinal mass, integument, skeleton and musculature were weighed on a platform balance, sensitive to the tenth of a gram. The other organs were weighed in closed containers on an analytical balance, the smaller organs being weighed to the tenth of a milligram.

In general, the organs were weighed immediately (by an assistant) and then preserved in 10 per cent formalin solution for histological study. In some cases portions were fixed also in Zenker's solution. From the formalin-fixed material, sections (5 micra) were made by the paraffin method, and stained with hematoxylin-eosin; or frozen sections (10–15 micra) were stained with hematoxylin-eosin or scarlet red (for fats). Paraffin sections of Zenker-fixed material were stained with hematoxylin-eosin.

Observations

In all cases the rats thrived upon the diet and were normal in appearance and behavior throughout the course of the experiments, which

extended from weaning up to nearly adult stage. No diarrhea or other evidence of gastro-intestinal disturbance was evident at any time. The growth in *body weight* was good, as shown by the curves in Fig. 1. Starting at 35 to 40 grams, the average weight of the males increased to 370 to 385 grams at 23 weeks of age, or after 20 weeks on the experiment. Although the sugar-fed male rats averaged slightly heavier toward the end of the period, they were not significantly different from the starch fed controls at any time. The female rats normally lag behind the males in body weight. In the present experiments they reached only about 250 grams average at 26 weeks of age. The females likewise showed no appreciable difference between the starch-fed (control) and the sugar-fed groups. The slightly lower body weight of the females on 80 per cent sugar diet toward the end of the experiment is probably explained by the fact that all these rats had recently completed a lactation period, which is known to have a somewhat depressing effect on the body weight.

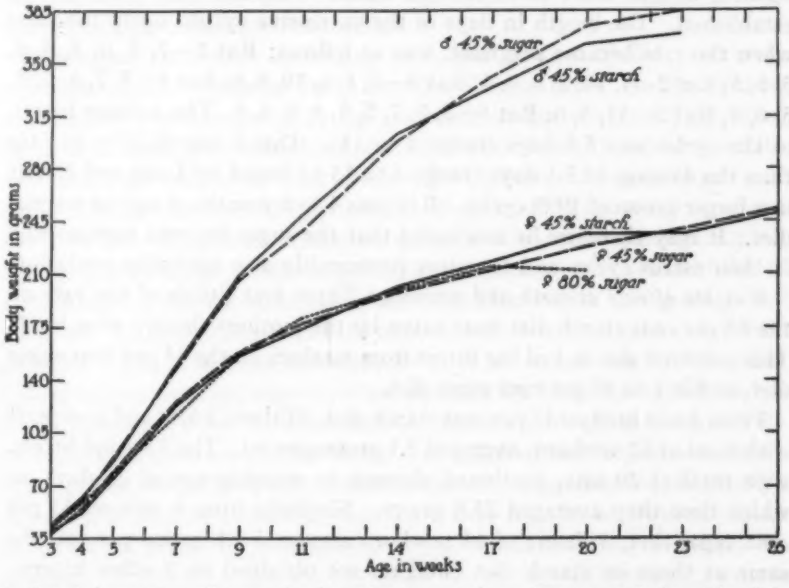

FIG. 1. Average growth curves for the various groups of rats. The greater body weight of the males is evident, but there is no significant difference between the starch-fed (controls) and the sugar-fed groups.

In general, it appears that the body weight of the Long-Evans strain of rats, both male and female, used in the present experiments is dis-

tinctly greater than the norm for the Wistar strain of albino rats at corresponding age, as given by Donaldson, 1924.

Reproduction: For the males, only two rats were tested from each of the first 2 groups. Of these rats, one in each case showed a positive test (normal vaginal plug and sperm), and one was negative on 2 or 3 trials. These observations are insufficient for conclusions; but, so far as they go, show no difference between the starch-fed and the sugar-fed rats. As will appear later the weights and histological appearances of the testis were normal.

Estrus cycles: In the 6 female rats fed 80 per cent sugar diet, the vagina opened at the average age of 55 days (range 47 to 66). This is considerably earlier than the average of 72 days (range 34 to 109) obtained by Long and Evans (1922) in rats of the same strain. We may therefore conclude that the high sugar diet had not retarded the associated maturity of the ovary. Vaginal smears were taken daily from the time the vagina opened, and in most cases indicated that ovulation was immediately established. The length in days of the successive cycles, up to the time when the rats became pregnant, was as follows: Rat 1—7, 5, 6, 5, 4, 6, 5, 5, 5; Rat 2—4, 10, 5, 6, 7, 4; Rat 3—5, 4, 6, 10, 8, 6; Rat 4—5, 7, 6, 6, 6, 5, 6, 4; Rat 5—11, 5, 6; Rat 6—5, 8, 7, 5, 5, 4, 5, 4, 5. The average length of the cycles was 5.8 days (range 4 to 11). This is but slightly greater than the average of 5.4 days (range 3 to 13+) found by Long and Evans in a larger group of 1999 cycles, all in rats 4 to 6 months of age on normal diet. It may therefore be concluded that the sugar-fed rats were normal in their estrus cycles and therefore presumably also normal in ovulation.

Weights of rats at birth and weaning: Three first litters of the rats on the 45 per cent starch diet were eaten by the mothers shortly after birth. This occurred also in 1 of the litters from mothers on the 45 per cent sugar diet, and in 1 on 80 per cent sugar diet.

From 3 rats bred on 45 per cent starch diet, 5 litters (2 first and 3 second) with total of 42 newborn, averaged 5.1 grams per rat. The 3 second litters, with total of 20 rats, continued through to weaning age of 21 days, at which time they averaged 25.8 grams. Similarly from 4 rats on 45 per cent sugar diet, 3 litters of 14 newborn averaged 5.1 grams per rat, the same as those on starch diet (weights not obtained on 2 other litters). The 5 litters, including 21 rats, reached weaning age with average of 33.5 grams, which was higher than those on starch diet. Two of these litters at weaning showed symptoms of hind limb paralysis. This was not observed in the 80 per cent sugar-fed group, however, and therefore cannot be ascribed to the sugar in the diet.

The 5 litters of newborn from rats on 80 per cent sugar diet totaled 28 individuals, with average weight of 5.1 grams. Of the 28 rats, 23 lived to weaning age of 21 days with average body weight 29.8 grams. This was above the average of weaning age for the starch-fed (control) group. In general, it is evident that the performance in reproduction and lactation was not very good in any of the groups. However, this often happens in the case of first litters. In any event it is clear that the rats on the high sugar diets were able to give birth to normal litters and to carry them through the lactation period with body weights above those from rats on the starch (control) diet.

Teeth: In view of the widespread opinion that excessive sugar diet is injurious to the teeth, these were carefully inspected during the course of the experiment as well as at autopsy. In most cases the teeth remained normal throughout the experiment. Occasional irregularities were noted on the incisors, with a few small carious spots on the molars; but these occurred with equal frequency in the starch-fed (control) and the test groups. It is therefore evident that high sugar diets for long periods are not necessarily injurious to the teeth.

Infections: Infections associated with pus in the middle ear cavity are often found in rats on stock or other normal diets. In the present experiment, pus was found in the tympanic cavity (on 1 or both sides) as follows; on 45 per cent starch diet, in 7 out of 15 (47 per cent) of the rats; on 45 per cent sugar diet, in 4 out of 14 (29 per cent); on 80 per cent sugar diet, in 4 out of 6 (67 per cent). On account of the small numbers, the high rate of incidence in the last group is of questionable significance. Combining all those on the sugar diets, pus occurred in 8 out of 20, or 40 per cent of the cases, as compared with 47 per cent in the starch-fed controls. No pus was found in the nasal fossae of any of the 6 rats fed 80 per cent sugar diet. The other groups were not examined on this point. No infections were apparent in other parts of the body, except the lungs. Lung infections occur in characteristic fashion with great frequency in rats on stock or other normal diets. The lungs of the 80 per cent sugar-fed rats were remarkably free from this common lung disorder. In general, however, it may be concluded that there is no significant difference as to incidence of pulmonary infections in the starch-fed and the sugar-fed groups.

Final body weight, body length and tail length: The mean absolute measurements for the body and tail (Table I) reveal no significant difference between the test rats and controls, excepting the 80 per cent sugar-fed group. The latter were slightly younger, with correspondingly smaller

TABLE I

AVERAGE AND RANGE OF MEASUREMENTS AT AUTOPSY FOR THE VARIOUS GROUPS OF RATS

Age in days; length in millimeters; body and organ weights in grams

	Male Rats		Female Rats		
	6 control rats (45% starch)	6 test rats (45% sugar)	9 control rats (45% starch)	9 test rats (45% sugar)	6 test rats (80% sugar)
Age	170 (153–177)	169 (149–185)	195 (178–216)	200 (177–227)	148 (143–160)
Nose-anus length	243.7 (235–256)	245.8 (234–259)	218.2 (210–226)	217.3 (209–230)	206.0 (201–214)
Tail length	215.0 (199–224)	212.5 (205–220)	195.4 (190–206)	192.7 (177–206)	189.4 (182–198)
Body weight	388.7 (322–458)	407.0 (337–466)	251.0 (232–278)	253.3 (215–300)	215.2 (197–230)
Head	24.7 (23.6–27.0)	25.2 (22.6–29.3)	19.7 (18.9–21.0)	19.0 (17.4–22.0)	16.9 (16.4–17.7)
Muscle and skeleton	186.2 (166–221)	190.3 (162–222)	128.0 (116–140)	122.7 (101–143)	102.8 (94.5–111)
Integument	80.9 (62–106)	81.4 (60–98)	47.2 (42–60)	45.7 (35–58)	35.1 (32–38)
Brain	1.982 (1.826–2.088)	1.968 (1.912–2.035)	1.815 (1.581–1.914)	1.825 (1.696–1.947)	1.808 (1.716–1.953)
Spinal cord	0.734 (.669–.828)	0.739 (.709–.793)	0.658 (.603–.731)	0.662 (.605–.715)	———
Eyeballs	0.304 (.291–.316)	0.319 (.305–.327)	0.300 (.282–.321)	0.293 (.261–.322)	0.277 (.261–.284)
Hypophysis	0.010 (.009–.011)	0.010 (.008–.012)	0.012 (.010–.017)	0.012 (.007–.016)	0.008 (.007–.008)
Thyroid gland	0.039 (.035–.042)	0.043 (.033–.053)	0.028 (.020–.043)	0.030 (.027–.034)	———
Thymus	0.197 (.148–.237)	0.171 (.123–.248)	0.156 (.092–.215)	0.156 (.116–.211)	0.202 (.085–.328)
Suprarenals	0.054 (.048–.057)	0.056 (.046–.071)	0.067 (.043–.086)	0.0649 (.045–.085)	0.052 (.043–.060)
Spleen	0.710 (.483–.841)	0.867 (0.615–1.427)	0.570 (.397–.653)	0.532 (.471–.697)	0.502 (.396–.689)
Heart	1.249 (.989–1.503)	1.304 (1.065–1.699)	0.892 (.750–1.100)	0.865 (.685–1.154)	0.773 (.676–.896)
Lungs	1.765 (1.163–2.161)	1.684 (1.317–1.930)	1.602 (1.183–2.286)	1.498 (1.166–2.300)	1.018 (.889–1.137)
Gastro intestinal mass (with contents)	25.1 (21.7–32.1)	24.1 (18.3–27.9)	19.3 (14.2–23.5)	18.9 (13.7–23.5)	19.6 (17.3–25.1)
Stomach (empty)	1.364 (1.152–1.440)	1.409 (1.148–1.587)	1.182 (0.952–1.388)	1.181 (0.919–1.754)	1.110 (.967–1.249)
Intestines (empty)	7.284 (5.687–8.881)	6.410 (5.189–7.355)	4.929 (3.800–5.949)	5.104 (3.933–8.340)	5.839 (5.071–6.889)
Tongue	0.953 (.935–.975)	0.953 (.818–1.035)	0.859 (.784–.935)	0.835 (.748–.953)	———
External orbital glands	0.194 (.176–.224)	0.174 (.162–.180)	0.151 (.110–.201)	0.143 (.092–.216)	———
Submaxillary glands	0.583 (.516–.626)	0.554 (.414–.689)	0.442 (.400–.499)	0.417 (.324–.623)	0.410 (.365–.451)
Pancreas	1.053 (.804–1.268)	1.000 (.933–1.106)	0.933 (.803–1.116)	0.943 (.723–1.213)	1.097 (.890–1.321)
Liver	15.19 (12.40–17.50)	16.60 (13.10–22.52)	9.52 (8.00–12.80)	10.48 (8.00–15.90)	12.19 (8.95–17.40)
Kidneys	3.080 (2.621–3.540)	3.407 (2.898–4.403)	2.052 (1.666–2.583)	2.245 (1.747–2.759)	1.985 (1.681–2.402)
Testes	2.912 (2.699–3.378)	3.058 (2.860–3.523)	———	———	———
Epididymides	1.149 (1.050–1.254)	1.147 (1.091–1.201)	———	———	———
Ovaries	———	———	0.065 (.049–.092)	0.070 (.054–.092)	0.063 (.047–.074)
Uterus	———	———	0.444 (.314–.565)	0.483 (.379–.619)	0.321 (.190–.436)

body weight and length, as well as smaller weights of the various organs and parts. The head weight likewise indicates no abnormality in the various groups.

Integument, muscle and skeleton: The integument shows no significant difference in weight between test and control groups. The fatty *tela subcutanea* (unweighed) likewise showed no abnormalities in amount or appearance. As previously explained under methods, the skeleton and musculature were weighed together. They appeared normal in all cases, with no significant difference in weights in the various groups.

Weights of the organs: As shown in Table I, there is generally a close agreement in weight of organs between the starch-fed (control) and the sugar-fed groups, making due allowance for the smaller size of the last (80 per cent sugar) group. This applies in general to the brain, spinal cord, eyeballs, thyroid gland, heart, gastro-intestinal mass, stomach and intestines, with or without contents (except last group), tongue, external orbital glands, pancreas (except last group), testes, epididymides, ovaries and uterus. Sex differences are apparent in the weights of the hypophysis and suprarenal glands.

In some cases there are apparent differences in weights, although usually of questionable significance. The weights of the hypophysis and (possibly) suprarenals appear relatively low in the last (80 per cent sugar-fed) group, while those of the intestines, pancreas and submaxillary glands are rather high. The larger size of the thymus in the last group agrees with the younger age. The spleen is normally subject to great individual variation. The low weight of the lungs in the last group is probably due to their unusual freedom from infection. The infection was, however, not very marked in any of the groups. The kidneys appear slightly (about 10 per cent) heavier in the test groups, male and female. The liver is also slightly heavier in the 45 per cent sugar-fed than in the starch-fed groups, both male and female. A more striking difference is seen in the last (80 per cent sugar-fed) group. In this group the liver, even with smaller body weight, is 28 per cent heavier than in the female starch-fed group. The significance of this increased weight of the liver will be apparent later, when the histology is considered.

Comparison with Wistar norms: Since the various groups of rats (especially the 80 per cent sugar-fed) differ more or less in average body weight, it is more accurate to compare the various organ weights with the normal for corresponding body length (or weight). The percentage differences of test and control animals can then be compared with each other, even though the body weights are different. While unfortunately no

norms are available for the Long-Evans strain, we may utilize the Wistar norms for this purpose (Donaldson, "The Rat," Prologue, pp. x-xi).

This procedure has been followed with the data from the present experiments, by comparing, for each individual rat, the percentage by which each organ weight differs from the Wistar norm at corresponding body length. The average percentages for each group were then computed. For economy of space the figures are not included in the table, but some of the results will be mentioned briefly.

In the first place, it is clear that the weights of some organs are distinctly different in the Long-Evans (present data for both test and control rats) and the Wistar strains at corresponding body weights or lengths. However, the numbers available in the present experiment are insufficient for statistical treatment with calculation of probable errors. Minor differences, below 10 per cent, will therefore be ignored, although they may possibly be significant (*e.g.*, the brain, which appears 3 to 6 per cent subnormal in all the present Long-Evans groups). Of the various organs, only the ovaries appeared distinctly above the Wistar norm (23 to 37 per cent). The other organs showing differences were below the Wistar norm. Sex differences were apparent in the eyeballs (which averaged 18 to 21 per cent subnormal in the males, but only 5 to 9 per cent in the females), and in the hypophysis (23 to 29 per cent subnormal in the males, 38 to 53 per cent in the females). The subnormality of other organs was as follows; submaxillary glands, 16 to 25 per cent (except last group); pancreas 31 to 43 per cent (except last group); spleen, 21 to 34 per cent; thyroid gland, 24 to 32 per cent (small number of cases!); heart, 11 to 19 per cent; lungs, 0 to 25 per cent (variable). The liver appeared 6 to 12 per cent subnormal in the male groups, and 23 to 28 per cent subnormal in the females, except the last (80 per cent sugar-fed) groups, which averaged 6 per cent above the Wistar norm.

If the data for the starch-fed and the sugar-fed groups are compared, in relation to the Wistar norm, the results are in general somewhat similar to those above-mentioned when the absolute weights (Table I) for the various groups were compared with each other. The results for the last group (with smaller body weight), however, can be more precisely determined by use of the norm. In this female group, fed 80 per cent sugar, in comparison with the female group fed 45 per cent starch, the weights appear relatively decreased in the hypophysis (15 per cent average) and the lungs (24 per cent, due to less infection?). On the other hand, the weights appear relatively increased in the submaxillary glands (11 per cent), kidneys (14 per cent), pancreas (24 per cent) and liver (34 per cent).

Some of these differences may not be significant, on account of accidental variations.

Histological observations: In the last (80 per cent sugar-fed) group formalin-fixed frozen sections were made of the liver in all 6, and of the liver, kidney, spleen, submaxillary glands, stomach, pancreas and thyroid gland (*in situ*, with larynx, pharynx, etc.) in 5. One set of the frozen sections for each organ was stained with hematoxylin-eosin; another set was stained with scarlet red for fats. For 1 rat of this group Zenker fixation and paraffin sections, stained with hematoxylin-eosin, were used on liver, kidney, spleen, submaxillary gland, stomach-duodenum, pancreas, thyroid gland (*in situ*), ovary, and uterus.

In the 4 groups fed 45 per cent starch or 45 per cent sugar, frozen sections of the liver were made in all 30, and paraffin sections of the testis in all of the 12 males. In 2 males (1 starch-fed and 1 sugar-fed) a nearly complete series of frozen sections was made, and in 6 others (2 males and 4 females, 3 starch-fed and 3 sugar-fed) thin paraffin sections were made (Zenker-fixed in 3 cases) of the gastro-intestinal tract, including liver, pancreas, submaxillary, external orbital gland, tongue, stomach, duodenum and large intestine. In only 1 or 2 cases each, sections were made also of the parotid gland, mammary gland, heart, lung, hypophysis, suprarenal gland, thymus and spinal cord. A more extensive study to be reported later, will be made of the kidney, suprarenal glands and hypophysis in this series of rats.

For the present it may be said that, with the exception of the liver, no significant difference in histological structure was found between the starch-fed controls and the sugar-fed test rats. Especial attention was given to the sections of the tongue, stomach and duodenum, but these appeared perfectly normal in all cases. Similarly the kidney, pancreas and submaxillary glands showed no histological peculiarities which might account for the above-mentioned differences in weight.

The liver: On account of the definite changes observed in the liver, the findings will be discussed more fully. As above mentioned, the liver appeared slightly heavier in the sugar-fed than in the starch-fed groups, especially in the 80 per cent sugar-fed in which the liver was distinctly hypertrophied. In most of the latter group, the liver at autopsy was noted as more or less yellowish in color, with distinct surface lobulation. In general, each lobule presented a thick, yellow periphery with a dark red center.

In histological sections of such livers, as shown in Fig. 2, each lobule presented a normal central area, surrounded by a wide peripheral zone

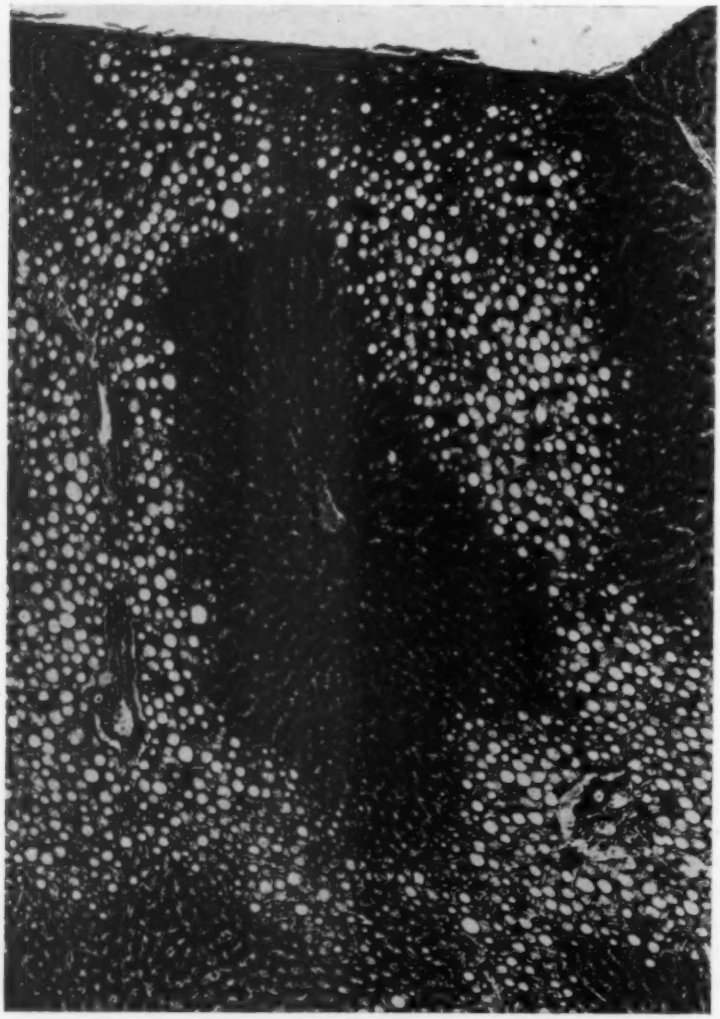

FIG. 2. From a photograph of a section of the liver (taken across the small three-sided lobe) in a female rat reared on a diet containing 80 per cent of sucrose. One lobule is shown, with normal central portion and central vein. The periphery of the lobule presents the characteristic structure, filled with fat droplets which appear as unstained vacuoles in the figure. The interlobular ducts and blood vessels are visible among the fat droplets. Zenker fixation; paraffin section; hematoxylin-eosin stain. X 95.

containing large vacuoles. These vacuoles, as shown by the scarlet red stains, are filled with fat droplets of variable size, which are all enclosed within the epithelial gland cells. In cases like that represented in Fig. 2 with wide peripheral fat zones completely surrounding the lobules, the fat was classified as "abundant." This occurred in 3 of the 6 livers of rats on the 80 per cent sugar diet, but in no other rat in any group. In 2 of the remaining 3 rats of the highest sugar-fed group, the fat cells were found in large groups around the interlobular vessels, but not entirely surrounding the lobules. In these cases the fat was classified as "moderate" in amount. In the remaining rat of the highest sugar-fed group, the fat was present in but small droplets in a few epithelial cells around the interlobular vessels, and was classified as "slight" in amount.

In the livers of the other 4 groups of rats, on 45 per cent starch or sugar, fat was likewise found in most cases, but in lesser amounts (Table II). In these cases, however, the fat was only once peripheral in position. Usually when it occurred it was diffusely scattered in cells throughout the lobules, in 3 cases only being centrally located. As appears from the distribution of cases shown in Table II, there is probably no sex difference in the occurrence of the fat, and no difference between the 45 percent starch-fed and the 45 per cent sugar-fed groups.

In sections under higher powers of the microscope, it appears that when the fat is small in amount the droplets are usually smaller, with variable numbers in a cell and varying in size from minute lipoidal granules up to moderate sized droplets. Where the fat becomes more abundant the smaller droplets tend to fuse into spheres much larger than the original gland cell within which they are contained, as is evident in Fig. 2. In such cells the nucleus is flattened and peripherally placed. In addition to these large fat droplets, smaller droplets and lipoidal granules occur in some of the cells. This is evident in sections with either stain, hematoxylin or scarlet red. It is remarkable that even in the sections stained with scarlet red, no lipoidal granules appeared except in areas associated with at least a few somewhat larger fat droplets, and no granules at all were found in the liver in cases shown in the first column of Table II.

The significance of the fat in characteristic abundance in the highest sugar-fed rats is difficult to explain. It can hardly be classified as "fatty degeneration," since neither the fat-containing cells nor any of the other liver cells show any degenerative abnormalities. Both nucleus and cytoplasm appear quite normal in structure, aside from the fat droplets. On the other hand, there is no evidence of any excessive fat elsewhere in the body, either in the *tela subcutanea* or in other regions, which might

suggest a general steatosis associated with fatty "infiltration" or deposit in the liver cells. It is clear that even with undoubtedly normal diets, there is often a slight tendency to deposition of fat in the liver cells. With the sugar up to concentration of 45 per cent of the diet this normal tendency was no greater than with diets in which the sugar was replaced with starch. But for some obscure reason when the diet contained 80 per cent sugar, with very much smaller amounts of fat, the tendency to fatty deposit in the liver cells appeared to be greatly increased. Finally, however, it must be admitted that the number of rats on the 80 per cent sugar diet was relatively small, and the results might possibly be due to an accidental variation. Further study on this point will therefore be made with a larger number of animals.

TABLE II

SHOWING THE DISTRIBUTION OF CASES AS TO THE AMOUNT OF FAT IN THE LIVER, AND ALSO THE LOCATION OF FAT IN THE LOBULE (p, PERIPHERAL; c, CENTRAL; d, DIFFUSE).

Group of rats	None	Slight	Moderate	Abundant
45% starch males	0	5 (all d)	1c	0
45% sugar males	1	4 (all d)	1d	0
45% starch females	4	5 (4d, 1c)	0	0
45% sugar females	4	4 (3d, 1c)	1p	0
80% sugar females	0	1p	2p	3p

In connection with the present work, it is of interest to recall that hepatic steatosis may be produced by various dietary means. Brüning (1914) found a fatty liver in rats on a one-sided carbohydrate diet of potatoes, sugar, "cakes" and water. Maignon and Jung (1922) similarly found that a diet of casein or fibrin produced an enlarged, yellowish liver, with marked fatty deposits in the periphery of the lobules. Jackson (1925) reviewed work showing that hepatic steatosis may occur in man and other animals under various dietary conditions, including overfeeding as well as inanition of various types.

DISCUSSION AND CONCLUSIONS

From the foregoing observations it may be concluded that the high sugar diets have no detrimental effect upon the growth and general health of rats. The various organs likewise show no abnormalities, aside from some few changes in relative weight, especially hepatic enlargement, and

(in the highest sugar-fed group) a tendency toward fatty deposits in the liver. But caution should be exercised in applying these conclusions to the human diet. Difference in species might render the rat more tolerant than man to large quantities of sugar.

Furthermore, while the present experiments show no direct injurious effects of high sugar diet in the rat, the indirect effects are tested only in part. It is evident that in the rat the appetite is not affected by the prolonged consumption of a high sugar diet. But under the conditions of the present experiments, an adequate intake of the other essential factors (protein, salts, fat, vitamins) was made certain. Such conditions are usually lacking in human nutrition. Therefore, even ignoring the possible difference in species as to tolerance of sugar, it is quite possible that with free choice of accessory-containing foods, a basal high-sugar diet might result in unbalanced nutrition with deficient intake of some of the essential factors. To exclude this possibility would require further experiments.

Summary

1. Thirty-six rats were reared from time of weaning to nearly adult stage on balanced diets containing 45 per cent starch (controls), 45 per cent sucrose or 80 per cent sucrose. The rats all remained normal in health, vigor and external appearance.

2. Growth in body weight was normal, with no significant differences between the starch-fed (control) and the sugar-fed groups.

3. Reproduction appeared practically normal in the females, as shown by the time of vaginal opening, the estrus cycles, birth of litters, and lactation performance.

4. At autopsy, no abnormalities were found in body length, tail length, or gross appearance of the various regions, parts, or organs, excepting the liver. The middle ears and lungs are subject to infections, which were not more frequent in the sugar-fed than in the starch-fed (controls).

5. In weight, most of the various parts and organs appeared normal and nearly equal in the starch-fed (control) and sugar fed groups. Differences (usually of somewhat questionable significance) included, especially in the 80 per cent sugar-fed group, an apparent decrease in average weight of the hypophysis, and an increase in the intestines, kidneys, submaxillary glands, pancreas and (especially) the liver.

6. Incidentally it was found that in general the Long-Evans strain of pied (hybrid) rats, which was used in the present experiments, differs markedly from the Wistar norm in weight of the body and of certain organs.

7. A careful histological study of the various organs in both starch-fed and sugar-fed rats revealed no significant abnormality of structure, excepting in the liver.

8. The liver in the 80 per cent sugar-fed group was enlarged, with (usually) more yellowish color and distinct surface lobulation. In histological sections most of this group showed marked fatty deposits in the glandular epithelium at the periphery of the lobules. In the other groups slighter amounts of fatty change frequently appeared, but more irregular in location, with no difference between the starch-fed and sugar-fed groups.

9. Caution should be exercised in applying the results of the present experiment to human diets.

Addendum. Since the completion of this study, an additional series of 20 adult test rats (4 Long-Evans and 16 of Wistar albino strain) was examined. These had been reared on similar balanced diets containing varied amounts of sugar. These rats showed no tendency to enlargement of the liver in any of the groups. The number of cases with corresponding distribution of fat in the liver was as follows: on 45 per cent sugar diet, 2 none (no fat); on 60 per cent sugar diet, 3 none, 1 slight, 1 moderate; on 72 per cent sugar diet, 1 none, 2 slight, 1 moderate; on 80–84 per cent sugar diet, 2 none, 2 slight, 4 moderate, 1 abundant. Combining this with the previous series, giving a total of 56 rats, the distribution may be summarized as follows: on 45 per cent starch diet, 4 none (27 per cent), 10 slight (67 per cent), 1 moderate (6 per cent); on 45–60 per cent sugar, 10 none (45 per cent), 9 slight (41 per cent), 3 moderate (14 per cent); on 72–84 per cent sugar, 3 none (16 per cent), 5 slight (26 per cent), 7 moderate (37 per cent), 4 abundant (21 per cent). The enlarged series still indicates a greater tendency to hepatic fat associated with higher percentages of sugar in the diet, although the fat appears abundant in only 21 per cent of even the most highly sugar-fed group.

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THE INFLUENCE OF HIGH AND LOW PROTEIN DIET ON THE BASAL METABOLISM AND THE CHEMISTRY OF BLOOD AND URINE IN NORMAL WOMEN

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A REVIEW of the literature revealed that the majority of the papers reported dealing with the relation of the protein intake to metabolism was usually made on a single subject with little attention directed to the caloric value of the various diets. In most cases the periods of the experiments were not systematically planned and the diets were abnormally unbalanced. An investigation was therefore conducted on a group of normal adult women with a carefully planned diet for a comparatively long time allowing a sufficient interval between periods for the body to become adjusted to the conditions of the experiment.

Experimental

In this study 6 apparently normal women, varying in ages between 17 and 36 years, all engaged in laboratory work, served as subjects for a total number of 81 days. The weight of the subjects ranged from 72.2 to 46.5 kilos and the percentage deviation from the standard (1) was from +9.4 to −18.1. For a period of 44 days they received a high protein diet containing 2 gm. of protein, and sufficient carbohydrate and fat to make 40 calories, per kilo. of body weight. An interval of 3 weeks was then allowed in which the protein intake was lowered until it reached 0.5 gm. per kilo. of body weight. Although the carbohydrate and fat content of the low protein diet was adjusted so that the caloric value of the two diets remained the same, all the subjects lost weight steadily for the first ten days during the low period. As several of the subjects became alarmed and insisted on giving up the diet, the protein intake was increased to 0.6 gm. per kilo. The loss of weight immediately ceased and the body weight of the six subjects remained stationary throughout the low protein period which lasted for 30 days. A month later, after the body had become readjusted to its normal conditions, the foods of the subjects were again carefully weighed for two weeks. During this period, which served as a control, a large variety of each class of foods was prepared and the subjects ate the kind and the quantity of foods they desired. All the essential food

constituents were well supplied throughout this investigation. A typical day's dietary included the following foods; milk, meat or cheese, egg, potato, bread, sugar, butter, three or more fresh vegetables and two or more fresh fruits. In the low protein diet the amount of milk was decreased from 22.0 grams, as in the high protein diet, to 6.5 grams per kilo. of body weight and 40 per cent cream was substituted for meat or cheese. Oranges predominated among the fresh fruits, which included apple, banana, cantaloupe, grapes, peach, and strawberries. The fresh vegetables consisted of beans, peas, celery, lettuce, radishes, cabbage, spinach and cauliflower. No glandular organs were used at any time. The calcium content of the foods amounted to approximately 0.34 grams per kilo. of body weight in the high protein diet. The corresponding value for the low protein diet was 0.18 grams.

TABLE I

COMPOSITION OF FOOD (WEEKLY AVERAGE) IN GMS.

Subject	Cal. per kg.			Prot. per kg.			Fat per kg.			Carb. per kg.		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
High Protein Diet												
I	41.2	40.6	40.7	2.0	2.0	2.0	2.1	2.0	2.0	3.6	3.6	3.6
II	42.5	40.4	41.1	2.0	2.0	2.0	2.2	2.0	2.1	3.6	3.6	3.6
III	41.9	41.2	41.4	2.1	2.0	2.1	2.1	2.0	2.1	3.7	3.7	3.7
IV	43.4	41.4	42.0	2.1	2.1	2.1	2.2	2.0	2.1	3.7	3.7	3.7
V	42.9	40.6	41.3	2.1	2.0	2.0	2.2	2.0	2.1	3.7	3.6	3.6
VI	40.8	40.1	40.3	2.0	2.0	2.0	2.1	2.0	2.0	3.6	3.6	3.6
Normal Diet												
I	21.2	19.0	20.1	0.6	0.6	0.6	1.1	1.0	1.1	2.2	1.8	2.0
II	40.5	35.8	38.2	1.5	1.2	1.4	2.2	1.8	2.0	3.7	3.7	3.7
III	33.7	33.6	33.7	1.2	1.2	1.2	2.0	1.8	1.9	3.2	2.8	3.0
IV	47.8	45.8	46.8	1.6	1.6	1.6	2.8	2.5	2.7	4.2	4.1	4.2
V	36.8	40.8	38.8	1.4	1.3	1.4	2.4	2.0	2.2	3.4	3.3	3.4
VI	35.7	30.9	33.3	1.1	1.1	1.1	2.1	1.7	1.9	3.2	2.8	3.0
Low Protein Diet												
I	40.8	39.0	40.3	0.6	0.5	0.6	2.5	2.4	2.4	4.2	3.5	4.0
II	41.0	38.9	40.4	0.6	0.5	0.6	2.5	2.4	2.4	4.2	3.5	4.0
III	42.0	40.1	41.5	0.7	0.5	0.6	2.6	2.4	2.5	4.3	3.6	4.1
IV	43.2	41.0	42.6	0.7	0.5	0.6	2.7	2.5	2.6	4.4	3.6	4.2
V	41.5	39.5	40.9	0.6	0.5	0.6	2.6	2.4	2.5	4.3	3.5	4.1
VI	40.8	39.0	40.3	0.6	0.5	0.6	2.5	2.4	2.4	4.2	3.5	4.0

Four days of each week during the high and low protein periods samples of foods were collected and a composite sample of aliquot portions of each was dried, pulverized and analyzed for total nitrogen by the Kjeldahl method. In the case of the control period each food was analyzed separately. Table I gives the weekly average of caloric, protein, fat and carbohydrate values of the high, normal and low protein diets calculated according to Rose's table. Of the 6 subjects, 5 had a lower caloric intake than 40 calories per kilo. of body weight during the normal diet. The unusually low intake of subject I and high intake of subject IV were partly due to their mental attitude toward the problem as both of them had become tired of living on a weighed diet. It is of interest to note that with the exception of subject I the protein intake in the control period fell between the high and low values.

Basal Metabolism

On two consecutive mornings of each week the heat production of each subject was measured by the Tissot gasometer in connection with the Haldane Gas Analysis Apparatus, as described in a previous publication (2) from this laboratory. All the tests were made under post-absorptive conditions and after a rest period of 30 minutes.

Out of the 132 tests, only 8 gave a higher basal heat production than the standards given by Harris and Benedict. The average deviation from the standards of each subject was −9.1, −9.7, −6.9, −6.0, −11.5 and −10.5 per cent during the high protein diet. The corresponding values for each subject during the normal diet were −2.4, −9.7, −2.2, +0.2, −4.0, and −6.9 per cent and those for the low protein period were −5.9, −11.9, −4.7, −6.5, −8.9, and −11.0 per cent.

The results in Table II are expressed in calories per square meter of body surface per hour as well as calories per kilo of body weight per hour. Contrary to the findings of Kleitman (3), Deuel and his associates (4) and of Wishart (5), no marked difference in the basal metabolic rate of our subjects was found during the three diets. In 5 of the 6 subjects during the period on control diet, the heat production was slightly higher, which might be due in part to the physical improvement of the subjects after a summer vacation, or to the difference in the temperature of the season, since this period occurred in October whereas the other two were in July and August. Hafkesbring and Collett (6) demonstrated an increase of about 5 per cent in the heat production of their two woman subjects in cold weather. Benedict and MacLeod (7) also pointed out that the heat production of their rats was lowered in the summer. The discrepancy

TABLE II

BASAL METABOLISM (WEEKLY AV.)

Subject	Wk.	Wt. kg.	Pulse	R. Q.	Cal. per sq. m. per hr.	Cal. per kg. per hour	Subject	Wk.	Wt. kg.	Pulse	R. Q.	Cal. per sq. m. per hr.	Cal. per kg. per hour
High Protein Diet													
	1	73.2	68	0.772	36.0	0.93		1	53.2	67	0.804	35.8	1.02
	2	73.3	72	0.765	32.5	0.84		2	53.2	65	0.792	33.9	0.99
I	3	74.3	83	0.777	34.0	0.87	IV	3	53.1	66	0.763	34.6	1.01
	4	73.7	78	0.773	34.1	0.88		4	53.6	67	0.768	34.0	0.99
	5	74.3	71	0.773	31.6	0.81		5	53.1	65	0.790	34.1	0.99
Av.		73.6	74	0.772	33.6	0.87	Av.		53.2	66	0.783	34.3	1.00
	1	60.9	65	0.819	34.0	0.93		1	51.8	76	0.832	32.7	0.98
	2	61.0	69	0.788	34.9	0.96		2	51.8	72	0.809	32.1	0.96
II	3	61.8	65	0.759	32.5	0.89	V	3	52.4	72	0.773	33.4	0.99
	4	61.0	65	0.783	33.4	0.92		4	51.8	69	0.796	32.9	0.99
	5	62.2	62	0.793	32.4	0.88		5	52.0	63	0.756	30.6	0.91
Av.		61.4	65	0.788	33.4	0.92	Av.		52.0	71	0.793	32.3	0.97
	1	57.5	72	0.829	34.8	0.97		1	47.3	62	0.744	34.5	1.03
	2	57.8	79	0.816	38.1	1.05		2	48.2	61	0.757	33.4	0.99
III	3	57.6	79	0.792	37.3	1.03	VI	3	47.7	66	0.748	34.7	1.03
	4	58.1	79	0.791	33.7	0.96		4	47.8	59	0.748	30.4	0.90
	5	58.1	76	0.807	34.2	0.97		5	48.2	60	0.728	30.4	0.90
Av.		57.8	77	0.807	35.6	1.01	Av.		47.8	63	0.745	32.7	0.97
Normal Diet													
I	1	71.8	80	0.741	34.0	0.89	IV	1	53.3	68	0.773	37.2	1.08
	2	70.7	71	0.743	38.3	1.00		2	52.9	72	0.768	36.0	1.05
Av.		71.3	76	0.742	36.2	0.95	Av.		53.1	70	0.771	36.6	1.07
	1	60.8	63	0.808	34.8	0.95		1	51.2	68	0.764	33.9	1.02
II	2	61.0	63	0.776	32.0	0.88	V	2	51.7	69	0.774	36.2	1.08
Av.		60.9	63	0.792	33.4	0.92	Av.		51.5	69	0.769	35.1	1.05
	1	55.8	76	0.736	36.0	1.02		1	47.5	53	0.718	33.3	0.99
III	2	56.7	71	0.773	38.4	1.07	VI	2	47.3	56	0.751	34.7	1.04
Av.		56.3	74	0.755	37.2	1.05	Av.		47.4	55	0.735	34.0	1.02

TABLE II (*Continued*)

BASAL METABOLISM (WEEKLY AV.)

Subject	Wk.	Wt. kg.	Pulse	R. Q.	Cal. per sq. m. per hr.	Cal. per kg. per hour	Subject	Wk.	Wt. kg.	Pulse	R. Q.	Cal. per sq. m. per hr.	Cal. per kg. per hour
					Low Protein Diet								
	1	72.1	69	0.757	33.4	0.87		1	51.1	64	0.758	32.9	0.97
	2	71.9	72	0.723	35.7	0.93		2	50.7	63	0.761	33.2	0.99
I	3	72.1	71	0.781	35.1	0.92	IV	3	50.6	67	0.708	35.9	1.07
	4	72.1	74	0.923	35.4	0.92		4	50.8	69	0.769	34.5	1.03
Av.		72.1	72	0.796	34.9	0.91	Av.		50.8	66	0.749	34.1	1.02
	1	60.4	61	0.777	31.2	0.85		1	51.2	62	0.785	33.7	1.01
	2	60.5	62	0.809	31.9	0.87		2	50.8	64	0.816	32.8	0.99
II	3	60.6	62	0.757	33.5	0.92	V	3	50.7	69	0.736	33.5	1.01
	4	60.1	65	0.766	33.9	0.94		4	50.7	68	0.740	33.0	1.00
Av.		60.4	63	0.777	32.6	0.90	Av.		50.8	66	0.769	33.3	1.00
	1	56.6	80	0.750	34.6	0.97		1	46.6	57	0.712	32.2	0.97
	2	56.4	75	0.755	37.7	1.05		2	46.5	69	0.722	32.4	0.98
III	3	56.4	86	0.785	38.3	1.07	VI	3					
	4	56.7	76	0.753	34.4	0.96		4	47.1	61	0.693	32.9	0.99
Av.		56.5	79	0.761	36.3	1.01	Av.		46.7	62	0.709	32.5	0.98

between our results and those of other investigators may be due partly to the variation of their caloric values, to the short duration of their high protein period, or to the abnormally large quantities of protein ingested by their subjects, especially in Kleitman's work. His subjects consumed as much as 4 pounds of meat daily for nine days or 36 to 48 egg whites per day which amounted to about 2 to 6 grams of protein per kilo. of body weight as compared with our 2 grams per kilo. In his investigation there was a large range in the caloric value of the different diets. This also holds true in the work reported by Deuel and his associates. Thus, the caloric value was constant during the low protein diet but when the protein was increased from 0.019 to 3.1 grams of nitrogen, the caloric value was more than doubled, increasing from 1830 to 3893 calories. The duration of his experimental periods too varied widely. The protein-free diet lasted 30 days while the subsequent periods were less than two weeks. The highest protein diet, containing about 3 grams per kilo.

of body weight, was only of 3 day's duration. With the exception of the low-protein diet, the periods were so short that the body did not have sufficient time to readjust itself to a constant level. In regard to the effect of the difference in caloric intake on basal heat production, Lusk (8) in his review of Benedict's experiments on the Y. M.C.A. students, recalculated some of the results, paying special attention to the relationship between the loss of body nitrogen and the reduction in basal metabolism. He pointed out that there was a striking discrepancy between the marked reduction in heat production and the comparatively insignificant loss of body nitrogen. He concluded, therefore, that there is not only a mechanism of nitrogen minimum protecting life from destruction, but also a biological adaptation to a lowered energy intake, preventing the exhaustion of the reserve of body fat. On account of insufficient data given by Wishart, we are unable to explain the discrepancy between his work and ours. In a study of Polar and Baffin Island Eskimos, Heinbecker (9) found that the basal metabolism of his subjects was considerably higher than that of persons living in the temperate zones. As was suggested by the author, this increase may probably be due to the climate.

Our results are in accord with those reported by Borgstrom and his associates (10), Hicks (11) and Lieb(12). The latter investigator, who made an extensive metabolic study on two explorers who lived on an exclusive fat-meat diet for 12 months, found no appreciable variation in the basal metabolism of his subjects. The values varied from −10 to −20 per cent by the Aub-DuBois standards. This work confirmed his earlier (13) study of Stefansson, and Asher and Honda (14) also found only a slight, if any, increase in the metabolism of rats fed on meat.

Blood Chemistry

Following the basal metabolism test while the subjects were still resting, blood was drawn from the cubital vein without a tourniquet and was analyzed for total non-protein nitrogen, urea nitrogen, creatinine, creatine, uric acid, sugar, lactic acid and calcium. On days when sufficient blood was drawn the icterus index was also estimated. With the exception of the total non-protein nitrogen, which was determined according to Koch's method (15) the nitrogenous constituents were analyzed by the Folin-Wu procedures (16). Clausen's method modified by Friedemann (17) was employed for lactic acid. Calcium was determined by the procedure of Kramer and Tisdall (18) and the icterus index by Brown's method (19). All determinations were made in duplicate and the values were expressed as mgs. per 100 cc. of whole blood with the exception of calcium

and icterus index for which serum was used. The values of calcium were given as mgs. per 100 cc. of serum.

Table III gives the weekly average values of the different blood constituents of the 6 subjects while they were on the three different diets. The total non-protein nitrogen varied between 37.0 and 66.7 mgs. on the high protein diet, as compared with 31.7 and 37.9 mgs. on the normal, and 27.3 and 31.8 mg. on the low protein diet. The averages of the subjects for the three periods were 47.1, 34.8 and 29.7 mgs. for the high, normal and low protein diets respectively. The urea nitrogen was low as compared with the non-protein nitrogen throughout the high protein period, varying from 13.0 to 20.6. The corresponding values for the normal and low protein diets were from 13.0 to 17.9 and 9.0 to 10.9 mgs. The averages of urea nitrogen for the three periods were 15.6, 15.6, and 9.7 mgs. respectively. Contrary to the findings of the high values of the total non-protein and urea nitrogen in the high protein period, the diet had little or no effect on either the uric acid or creatinine content. The average values of uric acid were 3.5 for the high, 3.2 for the normal, and 3.4 mg. for the low protein diet.

The creatinine content of the three groups was unusually constant, the average of each being 1.4 mgs. Creatine, like the total non-protein nitrogen, varied with the protein intake. The average values were 3.1, 2.6, and 2.2 mgs. for the high, normal and low protein periods respectively.

In contrast with the nitrogenous constituents, there was a tendency toward a higher value of sugar and lactic acid during the low protein period than in the high or normal periods. The average sugar values were 83.4 for the high, 82.5 for the normal, and 86.9 for the low protein diet, while those of lactic acid were 16.8, 19.4 and 23.9 mgs. respectively. The high carbohydrate content of the low protein diet may account for the high values of sugar. Since the well known theory that lactic acid is one of the intermediates of carbohydrate metabolism has recently been questioned, we are unable to account for the high lactic acid value during the low protein diet. However, Collazo and Morelli (20) also found that carbohydrates increased the lactic acid content of the blood. They demonstrated further that protein augmented the blood lactic acid but fat lowered it.

Diets, varying chiefly in the amount of protein, had no influence on the calcium content of the fasting blood, the average values being 9.7 mgs. for the high, 10.2 mgs. for the normal, and 9.4 mgs. for the low protein period. In the course of the calcium determination it was observed that the color of the serum was much darker than normal. Icterus index was therefore

TABLE III

WEEKLY AVERAGE OF BLOOD CHEMISTRY EXPRESSED AS MGS. PER 100 CC.

Subject	High Protein Diet						Normal Diet			Low Protein Diet				
	Weeks						Weeks			Weeks				
	1	2	3	4	5	Av.	1	2	Av.	1	2	3	4	Av.
	Total Non-protein Nitrogen													
I	51.4	55.2	66.7	45.4	40.7	51.9	32.4	35.0	33.7	29.2	29.1	29.1	31.1	29.6
II	51.3	47.4	54.3	45.5	43.8	48.5	37.9	32.5	35.2	29.9	28.4	30.2	30.8	29.8
III	43.5	51.2	47.6	37.0	39.6	43.8	31.7	36.1	33.9	38.4	28.4	30.2	29.2	29.1
IV	43.5	44.9	52.3	41.8	41.6	44.8	34.6	37.6	36.1	28.5	29.3		31.8	29.9
V	45.9	41.7	57.2	46.4	39.7	46.2	34.3	34.7	34.5	28.2	27.5	29.8	30.9	29.1
VI	51.2	56.1	46.5	41.8	41.7	47.3	33.5	36.8	35.2	31.8	28.7		31.8	30.8
	Urea Nitrogen													
I	18.6	14.5	16.4	15.9	13.6	15.8	16.7	16.3	16.5	9.0	9.9	9.6	10.1	9.7
II	19.9	16.0	14.8	15.8	14.2	16.1	15.1	13.4	14.3	9.3	9.7	9.1	9.4	9.4
III	16.2	15.4	14.0	13.0	13.9	14.5	17.0	15.7	16.4	9.5	10.0	10.0	10.9	10.1
IV	15.9	14.3	15.0	14.9	13.7	14.8	13.0	15.1	14.1	9.1	9.8		9.4	9.4
V	20.6	15.1	14.2	17.1	15.3	16.5	17.9	17.3	17.6	9.4	9.8	9.1	10.8	9.8
VI	1.85	17.7	15.4	14.8	14.2	16.1	13.7	15.1	14.4	9.6	9.7		10.0	9.8
	Uric Acid													
I	3.9	4.3	4.0	3.6	3.2	3.8	3.1	3.8	3.5	3.4	3.1	3.2	3.3	3.3
II	3.2	3.1	3.4	3.4	3.2	3.3	2.9	2.7	2.8	3.4	3.2	3.2	3.6	3.4
III	3.3	3.3	4.3	3.3	3.3	3.5	3.2	3.7	3.5	3.5	3.1	3.5	3.3	3.4
IV	3.3	3.1	3.2	3.5	3.2	3.3	2.7	4.1	3.4	3.4	3.2		3.3	3.3
V	3.6	3.8	3.6	3.5	2.9	3.5	2.5	2.5	2.5	3.6	3.1	3.2	3.2	3.4
VI	4.8	3.3	4.3	3.5	3.2	3.8	3.8	3.2	3.5	3.9	3.1		3.5	3.5
	Creatinine													
I	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.4	1.3	1.3
II	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
III	1.4	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4	1.3	1.4	1.4	1.4
IV	1.3	1.3	1.4	1.4	1.4	1.4	1.3	1.4	1.4	1.3	1.4		1.4	1.4
V	1.4	1.4	1.3	1.4	1.3	1.4	1.4	1.3	1.4	1.4	1.4	1.4	1.3	1.4
VI	1.2	1.3	1.2	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4		1.4	1.4
	Creatine													
I	2.9	3.0	3.3	3.0	3.0	3.1	2.6	2.3	2.5	2.2	2.2	2.1	2.2	2.2
II	2.6	2.9	3.2	3.2	3.0	3.0	2.6	2.2	2.4	2.1	2.1	2.2	2.3	2.2
III	3.0	3.0	3.2	3.3	3.0	3.1	2.7	2.6	2.7	2.2	2.2	2.3	2.3	2.2
IV	2.5	2.9	3.0	3.2	3.0	2.9	2.5	2.4	2.5	2.1	2.0		1.9	2.0
V	2.7	3.1	3.1	3.2	3.1	3.0	2.6	3.2	2.9	2.3	2.0	2.2	2.2	2.2
VI		3.2	3.1	3.3	3.2	3.2	2.7	2.9	2.8	2.3	2.1		2.1	2.2

TABLE III (*Continued*)
WEEKLY AVERAGE OF BLOOD CHEMISTRY EXPRESSED AS MGS. PER 100 CC.

Subject	High Protein Diet						Normal Diet			Low Protein Diet				
	Weeks						Weeks			Weeks				
	1	2	3	4	5	Av.	1	2	Av.	1	2	3	4	Av.
	Sugar													
I	85.2	86.4	88.5	88.1	86.4	86.9	84.4	86.0	85.2	84.4	89.5	82.1	84.2	85.0
II	77.1	84.0	90.1	81.1	80.3	82.5	76.9	77.4	77.2	88.9	89.3	82.7	78.6	86.4
III	81.4	80.9	77.8	81.5	84.1	81.1	83.7	82.8	83.3	87.9	89.1	89.3	87.4	88.4
IV	84.3	83.8	95.7	84.0	83.5	86.3	91.1	86.6	88.9	85.5	88.9		85.5	86.6
V	78.4	78.0	79.9	83.3	84.9	80.9	77.4	75.9	76.7	83.0	86.5	82.1	82.3	83.5
VI	76.5	82.6	87.9	83.7	82.0	82.5	83.7	8.30	83.4	97.3	88.1		89.5	91.6
	Lactic Acid													
I	18.0	18.3	19.5	17.3	21.7	19.0	22.5	16.2	19.4	18.1	20.5	21.9	25.1	21.4
II	15.2	16.6	19.2	17.3	20.0	17.7	21.2	18.7	20.0	22.0	23.5	25.6	22.2	25.8
III	13.7	17.2	19.0	13.6	20.3	16.8	20.7	17.0	18.9	19.5	21.9	24.7	24.0	22.5
IV	11.5	12.4	20.0	12.8	17.3	14.8	20.2	17.5	18.9				23.5	23.5
V	8.8	15.8	20.7	12.8	17.0	15.0	18.3	19.5	18.9	21.7	21.9	24.2	26.2	23.5
VI	12.0	19.0	18.9	14.5	18.9	17.7	22.5	18.3	20.4	24.6	25.3		29.4	26.4
	Calcium													
I	10.6	10.8				10.7	10.2		10.2	10.5	9.7			10.2
II	9.4	9.0				9.2	9.8		9.8	10.2	10.1			10.2
III	10.3	9.6				10.0	10.5		10.5	10.0	7.7			8.9
IV	10.4	9.9				10.2	10.7		10.7		9.0			9.0
V	8.4	8.9				8.7	10.3		10.3	9.4	9.1			9.3
VI	9.1	9.7				9.4	9.4		9.4	10.2	7.3			8.8

determined and in every subject the value was much higher than normal. The average of all the subjects for the high protein period was 11, while that of the low protein period was 8. The high values were undoubtedly due to the coloring matter in the foods, especially in oranges, carrots and eggs which were used extensively in the diets. This also may account for the higher value obtained during the high protein diet as more eggs were consumed during this period.

Papers dealing with the chemistry of the blood in relation to diet are extremely few and scattered. Both Jackson and Rigg (21) and Addis and MacKay (22) found a high total non-protein nitrogen in the blood of rats which were fed on abnormally high protein diet. Most investigators concluded that diet had no influence on blood and that the values were

within normal limits. However, none of them has done sufficient work to make a comparison of the values when the same subjects were on different levels of protein intake. In a study of the minimum endogenous nitrogen metabolism made on a medical student, Smith obtained a total non-protein nitrogen value of 35 mgs. per 100 cc. of whole blood when the subject was on an unrestricted diet, as compared with 21 mgs. when the subject received 2.08 gms. of protein per day, thus giving a decrease of 40 per cent although both values were within normal limits. The uric acid values of his subject showed no marked variation while, similarly to ours, his sugar values increased from 102 to 118 mg. In the study of the nitrogen minimum conducted on one man, Deuel and his coworkers (4) reported a series of blood determinations made while the man was on an extremely low protein diet. No appreciable difference was found in the total non-protein nitrogen or in any of the other nitrogenous compounds. However, the protein intake during these days was always very low. His sugar values fluctuated between 87 and 100 mgs. In Lieb's (12) study of two Arctic explorers, and in Heinbecker's (9) study of three Eskimos, they found that the chemical composition of the blood was within normal limits. No figures were given by Lieb and in the study made by Heinbecker the values ranged between 29 and 43 mgs. although the determinations were made on filtrates which had a slight growth of mold. Since no study was made while the Eskimos were on a low protein diet, his results were not comparable to ours. In the study of the metabolism of two Arctic explorers living on an exclusive meat diet for 12 months under average conditions in a New York climate, Tolstoi (23) reported that with the exception of a lipemia and hypercholesterolemia, the chemical composition of the blood was little affected by such a diet. However, an examination of the tables shows that with one exception, which was 33 mgs., the values of total non-protein nitrogen varied from 38 to 43 mgs. in one subject and from 44 to 53 mgs. per 100 cc. of blood in the other. The corresponding values for urea nitrogen were 18 to 22 mgs. and 23 to 29 mgs. per 100 cc. of blood. The author claimed that the values determined before the experiment started were within the above limits. These values are undoubtedly high for subjects living on a normal or a low protein diet which generally results in a value within 35 mgs. per 100 cc. of blood. The reason for the failure of an exclusive fat-meat diet to raise the nitrogenous content of the blood in these two subjects may be the fact that these men probably had been living habitually on a high protein ration and the experimental diet did not contain much more protein than their customary intake, merely a substitution of fat for carbohydrate.

Blood Pressure

Along with the other studies in this investigation, blood pressure was taken weekly. In agreement with the findings of many other investigators, no relationship was found between the protein intake and blood pressure. The average value of all the subjects on the high protein diet was 102 systolic and 62 diastolic while the values for the normal and low protein diets were 105 systolic and 59 diastolic and 105 systolic and 61 diastolic.

Nitrogen Metabolism

Two twenty-four hour specimens of urine were collected on two consecutive days of each week throughout this investigation. Total nitrogen was determined by the macro Kjeldahl method. Urea, ammonia, creatinine, creatine, and uric acid were estimated by the usual methods of Folin.

Weekly averages, expressed as mgs. per kilo. of body weight per 24 hours, are given in Tables IV, V and VI. A summary of the three tables is given in Table VII. As has already been shown by many investigators, a high protein diet resulted in an increase of total, urea and ammonia nitrogen, creatine and uric acid. The urea nitrogen decreased with total nitrogen as the protein intake was lowered from 2 to 0.6 gm. per kilo of body weight. The average percentage of total nitrogen as urea nitrogen was 87.4 for the high protein period and 80.4 and 76.2 for the normal and low protein periods. The gradual decrease of the uric acid, resulting from lowering the protein intake, was undoubtedly due to the nature of the proteins used on the different diets. In the high protein diet approximately 35 per cent of the protein was derived from meat while in the normal diet there was a great daily variation in different individuals with an average ranging between 25 and 40 per cent. No meat was used in the low protein diet. Similarly to uric acid, the ammonia nitrogen decreased with the decrease of protein intake. The average values of the three periods were 7.4 for the high, 5.1 for the normal and 2.8 mgs. per kilo. of body weight for the low protein diet. When it is expressed in percentage of total nitrogen, the ammonia nitrogen varied inversely with the nitrogen intake. Thus the average value for the high protein diet was 3.1 per cent and those for the normal and low protein diets were 3.2 and 3.7 per cent respectively. A marked increase of creatinine output was observed in 2 of the 6 subjects, and a slight increase in one, during the high protein diet. The average values for the period were 20.7 mgs. per kilo. of body weight for subject I, 23.6 mgs. for subject IV, and 22.6 mgs. for subject V. The corresponding values for the low protein diet were 17.4, 20.9 and 21.9, respectively. The

increased creatinine excretion was probably due in part to the excessive amount of exercise undertaken by these subjects during this period. In fact, subjects I and IV had a walking race before meals, averaging more

TABLE IV

NITROGEN METABOLISM PER KILO. PER 24 HOURS (WEEKLY AV.) HIGH PROTEIN DIET

Wk.	N. Intake mgs.	Urinary Nitrogen								
		Total N. mgs.	Urea N.		Ammonia N.		Creatinine		Creatine mgs.	Uric acid mgs.
			Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Creat. Coef. mgs.		
Subject I										
1	327	257	213	82.5	7.9	2.8	20.7	7.7	5.0	11.5
2	337	270	245	91.0	7.1	2.6	20.8	7.7	3.9	9.3
3	319	254	227	89.4	7.9	3.2	20.6	7.7	4.6	10.0
4	329	253	224	88.6	7.7	3.0	20.5	7.6	3.8	7.9
5	320	246	215	87.4	7.1	2.9	20.9	7.8	6.4	9.5
Av.	326	256	225	87.8	7.4	2.9	20.7	7.7	4.7	9.6
Subject II										
1	313	253	223	87.9	7.2	2.8	20.3	7.6	5.0	12.4
2	341	276	228	82.9	8.3	3.0	20.5	7.6	5.0	12.8
3	346	283	236	83.3	8.6	3.0	20.1	7.5	5.7	12.4
4	325	272	254	93.6	7.5	2.7	20.5	7.6	5.5	11.8
5	308	255	233	91.2	8.5	3.3	20.5	7.6	5.7	9.3
Av.	327	268	235	87.8	8.0	3.0	20.4	7.6	5.4	11.7
Subject III										
1	333	283	220	77.6	7.7	2.7	22.3	8.3	6.8	13.4
2	345	261	225	86.3	7.5	2.9	22.1	8.2	4.8	11.4
3	324	270	231	85.4	8.5	3.1	22.3	8.3	4.3	11.4
4	331	193	180	91.4	7.4	3.8	21.5	8.0	4.0	9.3
5	328	262	228	86.8	5.5	2.1	22.1	8.2	2.0	10.3
Av.	332	255	217	85.5	7.3	2.9	22.1	8.2	4.4	11.2
Subject IV										
1	320	300	277	92.3	9.1	3.1	23.4	8.7	6.6	12.2
2	349	280	257	92.8	7.9	2.8	23.7	8.8	5.1	11.3
3	354	292	267	91.2	7.6	2.6	23.5	8.7	7.7	11.6
4	333	260	234	90.0	7.3	2.8	23.9	8.9	6.8	9.6
5	315	263	245	93.1	9.0	3.5	23.6	8.7	4.2	11.3
Av.	334	279	256	91.9	8.2	3.0	23.6	8.8	6.1	11.2

TABLE IV (*Continued*)
NITROGEN METABOLISM PER KILO. PER 24 HOURS (WEEKLY AV.) HIGH PROTEIN DIET

Wk.	N. Intake mgs.	Urinary Nitrogen								
		Total N. mgs.	Urea N.		Ammonia N.		Creatinine		Creatine mgs.	Uric acid mgs.
			Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Creat. Coef. mgs.		
					Subject V					
1	315	230	206	89.6	6.7	2.9	22.3	8.3	7.0	10.5
2	343	271	224	87.5	7.3	2.7	23.0	8.6	6.5	10.5
3	348	278	251	90.3	9.1	3.3	22.8	8.5	8.0	10.5
4	327	258	207	80.5	6.2	2.4	22.6	8.4	6.8	11.8
5	310	229	193	84.7	7.3	3.2	22.2	8.3	6.7	9.2
Av.	329	253	216	85.5	7.3	2.9	22.6	8.4	7.0	10.5
					Subject VI					
1	324	295	225	78.3	7.0	2.6	20.1	7.4	7.3	11.2
2	336	270	234	87.0	7.5	2.8	20.0	7.4	9.2	10.9
3	316	257	233	90.9	6.2	2.4	19.9	7.4	5.4	17.9
4	329	257	221	86.2	7.6	3.0	19.8	7.5	7.5	9.2
5	320	247	216	88.1	6.6	2.7	19.5	7.3	10.5	10.9
Av.	325	265	226	86.1	7.0	2.7	19.9	7.4	8.0	12.0

than ten miles a day. During the low protein diet they were tired of living on a prescribed ration and took no exercise of any kind. The effect of exercise on creatinine excretion was clearly demonstrated in the study made by Cathcart and Burnett (24) on the influences of muscle work on metabolism. With no exception, their subject showed an increase in the output of creatinine during the period of muscular exercise. The creatinine values of our other three subjects were quite constant, with an average of 20.4 mgs. per kilo. body weight for subject II, 22.1 for subject III and 19.9 for subject VI during the high protein period. The corresponding values for the low protein period were 20.3, 22.1 and 19.8 respectively. The average creatinine coefficients of all the subjects for the high, normal and low protein periods were 8.1, 7.9 and 7.8 mgs. of creatinine nitrogen per kilo. of body weight. These values are in close agreement with those reported by McLaughlin and Blunt (25) in a study of a group of university women. They gave an average creatinine coefficient of 7.5 mgs.

Assuming 10 per cent of the food nitrogen as fecal nitrogen, a positive balance was found in all except subject III during the low protein period.

TABLE V

NITROGEN METABOLISM PER KILO. PER 24 HOURS (WEEKLY AV.)
Normal Diet

Wk.	N. Intake mgs.	Urinary Nitrogen								
		Total N. mgs.	Urea N.		Ammonia N.		Creatinine		Creatine mgs.	Uric acid mgs.
			Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Creat. Coef. mgs.		
Subject I										
1	104	116	92	79.7	3.4	2.9	19.2	7.2	2.1	7.1
2	98	99	75	75.7	3.9	3.9	19.2	7.1	1.9	6.5
Av.	101	108	84	77.7	3.7	3.4	19.2	7.2	2.0	6.8
Subject II										
1	236	181	154	85.1	4.7	2.6	20.4	7.6	2.3	11.3
2	211	148	112	76.2	6.0	4.1	20.5	7.6	2.1	9.9
Av.	224	165	133	80.7	5.4	3.4	20.5	7.6	2.2	10.6
Subject III										
1	200	166	137	82.5	4.4	2.6	21.2	7.9	2.3	9.8
2	213	170	134	78.9	6.1	3.6	22.2	8.2	2.3	9.8
Av.	207	168	136	80.7	5.3	3.1	21.7	8.1	2.3	9.8
Subject IV										
1	255	196	151	78.1	5.1	2.6	23.1	8.6	3.6	10.8
2	263	210	171	81.4	6.3	3.0	23.2	8.6	3.1	9.3
Av.	259	203	161	79.8	5.7	2.8	23.2	8.6	3.3	10.1
Subject V										
1	216	180	146	81.0	4.3	2.4	21.7	8.1	2.3	8.2
2	248	180	144	79.9	6.0	3.4	21.9	8.2	4.4	10.2
Av.	232	180	145	80.5	5.2	2.9	21.8	8.2	3.4	9.2
Subject VI										
1	176	161	128	79.6	5.3	3.3	19.8	7.4	4.0	8.8
2	179	161	138	85.9	5.6	3.5	19.7	7.3	4.7	8.9
Av	177	161	133	82.8	5.5	3.4	19.8	7.4	4.4	8.9

TABLE VI

NITROGEN METABOLISM PER KILO. PER 24 HOURS (WEEKLY AV.)
Low Protein Diet

Wk.	N. Intake mgs.	Urinary Nitrogen								
		Total N. mgs.	Urea N.		Ammonia N.		Creatinine		Creatine mgs.	Uric acid mg.
			Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Creat. coef. mgs.		
Subject I										
1	78	63	47	74.5	2.4	3.8	17.4	6.5	1.5	4.7
2	90	70	54	77.0	2.1	2.9	17.3	6.5	1.1	4.3
3	93	70	53	76.3	2.8	4.0	17.3	6.5	1.0	5.2
4	102	76	58	76.8	2.4	3.2	17.5	6.6	1.0	5.7
Av.	91	70	53	76.2	2.4	3.5	17.4	6.5	1.2	5.0
Subject II										
1	76	76	58	76.9	3.2	4.2	20.2	7.7	1.1	6.8
2	94	72	59	82.5	2.9	4.1	20.3	7.7	0.9	6.8
3	95	64	52	80.0	3.0	4.7	20.4	7.7	0.8	6.3
4	96	69	55	79.6	2.9	4.3	20.3	7.7	0.7	6.7
Av.	90	70	56	79.8	3.0	4.3	20.3	7.7	0.9	6.6
Subject III										
1	80	76	58	75.7	3.3	4.3	22.2	8.6	1.3	6.9
2	82	81	60	74.1	2.6	3.2	22.2	8.7	1.2	5.7
3	95	89	60	66.9	3.1	3.6	22.1	8.6	0.9	7.3
4	105	96	66	69.5	3.5	3.8	21.9	8.5	1.4	7.9
Av.	93	86	61	71.6	3.1	3.7	22.1	8.6	1.2	7.0
Subject IV										
1	80	89	70	78.5	3.2	3.6	21.0	8.3	2.1	6.1
2	93	73	58	79.3	2.1	3.0	20.9	8.3	1.1	5.2
3	100	70	52	74.1	3.2	4.6	20.9	8.4	1.1	5.8
4	101	79	59	75.5	2.6	3.3	20.8	8.3	1.2	6.2
Av.	94	78	60	76.9	2.8	3.6	20.9	8.3	1.4	5.8
Subject V										
1	79	74	61	81.8	3.0	4.0	21.7	8.5	2.1	5.4
2	95	85	65	76.5	3.2	3.7	21.9	8.4	2.9	6.7
3	96	82	66	79.9	2.7	3.3	22.0	8.5	1.8	5.3
4	97	86	64	75.1	2.7	3.1	21.8	8.3	1.8	5.9
Av.	92	82	64	78.3	2.9	3.5	21.9	8.4	2.2	5.8

TABLE VI (*Continued*)

NITROGEN METABOLISM PER KILO. PER 24 HOURS (WEEKLY AV.)
Low Protein Diet

Wk.	N. Intake mgs.	Urinary Nitrogen								
		Total mgs.	Urea N.		Ammonia N.		Creatinine		Creatine mgs.	Uric acid mgs.
			Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Per cent of total N.	Per 24 hrs. mgs.	Creat. coef. mgs.		
Subject VI										
1	78	76	60	78.4	2.5	3.3	19.8	7.5	1.6	6.1
2	90	78	58	74.2	2.3	2.9	20.0	7.6	1.5	5.6
3	97	81	53	61.0	3.0	3.7	19.7	7.5	1.7	5.1
4	102	71	59	83.1	2.0	3.3	19.7	7.4	0.8	6.4
Av.	92	77	58	74.2	2.5	3.3	19.8	7.5	1.4	5.8

TABLE VII
SUMMARY OF NITROGEN METABOLISM EXPRESSED AS MILLIGRAMS PER KILO. OF BODY WEIGHT PER 24 HOURS (WEEKLY AV.)

	High protein diet	Normal diet	Low protein diet
Nitrogen intake, mgms.	329	200	92
Total urinary nitrogen, mgms.	263	164	77
Urea nitrogen, mgms.	229	132	59
Urea nitrogen per cent in total	87.4	80.4	76.2
Ammonia nitrogen, mgms.	7.4	5.1	2.8
Ammonia nitrogen per cent in total	3.1	3.2	3.7
Fecal nitrogen (assumed), mgms.	32.9	20.0	9.2
Nitrogen balance, mgms.	+33.3	+15.8	+6.2
Creatinine, mgms.	21.6	21.0	20.4
Creatine, mgms.	5.9	2.9	1.4
Creatinine coefficient	8.0	7.9	7.8
Uric acid	11.0	9.2	6.1

She was only 17 years old and apparently still growing. This is also shown by the high retention of nitrogen by this subject during the high protein diet. The high retention of subject V during this diet may be explained by the fact that she was 16 per cent below the standard weight and was also decidedly underweight in appearance although she was physically normal. We are unable to explain the creatinuria which was present in all subjects throughout the low protein period containing no meat. However, it might be due to the mental and physical strain under which the subjects lived during this study, for they served as subjects in addition

to their regular laboratory duties. Furthermore, most of them lived several miles away from the institution.

Our low protein diet which contained 0.6 gm. of protein per kilo. of body weight resulted in an average nitrogen retention of 6.2 mgs. per kilo. of body weight. Such a diet was apparently low only in comparison with our high protein diet and high when compared with the diets used by Deuel and his associates (4) and Smith (26) who used a minimum protein diet resulting in a urinary nitrogen of 1.58 grams per 24 hours.

Urinary Acids

Total titratable, organic, and lactic acids were also determined on the specimens collected during these diets. The usual titrating method with phenolphthalein as indicator was employed for the determination of total acidity. The method of Van Slyke and Palmer (27) was used for the estimation of organic acids while Friedemann's procedure was employed in the determination of lactic acid. All the values as shown in Table VIII

Table VIII

Urinary Acids Expressed as cc. N/10 Acid per Kilo. per 24 Hours (Weekly Av.)

Subject	Total Titratable Acid						Organic Acid						Lactic Acid					
Weeks	I.	II.	III.	IV.	V.	VI.	I.	II.	III.	IV.	V.	VI.	I.	II.	III.	IV.	V.	VI.
High Protein Diet																		
1	5.8	5.8	5.0	7.8	3.6	8.2	6.9	6.5	8.8	8.3	6.8	8.0	0.2	0.3	0.3	0.4	0.2	0.3
2	5.6	6.3	4.7	6.2	6.0	8.1	6.8	7.4	8.1	9.0	7.2	7.9	0.3	0.3	0.3	0.5	0.4	0.4
3	5.5	7.4	5.5	6.7	6.1	8.1	7.2	7.4	7.0	8.7	7.4	7.6	0.4	0.4	0.3	0.4	0.5	0.4
4	8.6	8.8	7.0	6.6	7.7	9.2	6.6	9.1	7.1	9.7	8.9	7.4	0.3	0.2	0.3	0.5	0.3	0.4
5	6.7	7.9	6.0	8.1	7.7	7.0	6.7	8.8	9.6	10.0	7.5	7.7	0.2	0.3	0.4	0.4	0.3	0.3
Aver.	6.4	7.2	5.6	7.1	6.2	8.1	6.8	7.8	8.1	9.1	7.6	7.7	0.3	0.3	0.3	0.4	0.3	0.4
Normal Diet																		
1	5.5	6.0	5.7	6.5	5.9	6.0	5.1	7.2	5.7	8.2	6.2	7.0	0.2	0.3	0.3	0.3	0.3	0.2
2	4.1	5.3	6.5	7.6	5.2	6.3	4.5	7.0	6.8	8.1	7.1	6.8	0.2	0.3	0.2	0.2	0.2	0.3
Aver.	4.8	5.7	6.1	7.1	5.6	6.2	4.8	7.1	6.3	8.2	6.7	6.9	0.2	0.3	0.3	0.3	0.3	0.3
Low Protein Diet																		
1	2.9	4.3	2.5	3.9	2.7	2.6	6.1	6.9	6.1	8.6	7.5	7.7	0.3	0.3	0.3	0.4	0.3	0.2
2	2.2	3.3	2.6	3.3	3.3	2.9	6.4	6.7	6.0	6.5	7.5	8.6	0.4	0.3	0.3	0.3	0.2	0.2
3	2.9	3.1	3.0	2.8	2.5	2.9	5.4	6.6	5.9	8.1	7.4	7.1	0.2	0.3	0.3	0.3	0.3	0.4
4	3.3	3.1	3.2	3.7	2.8	2.5	5.5	7.9	7.1	8.7	8.0	7.1	0.3	0.4	0.3	0.4	0.3	0.3
Aver.	2.8	3.5	2.8	3.4	2.8	2.7	5.9	7.0	6.3	8.0	7.6	7.6	0.3	0.3	0.3	0.4	0.3	0.3

are expressed as cc. of N/10 acid per kilo. of body weight per 24 hours. As would be expected, the total titratable acidity decreased with a decrease in protein in all of the six subjects. However, there was an individual variation with a very slight tendency for the titratable acidity to increase with a decrease of body weight when it is calculated as cc. per kilo. of body weight. The average values were 6.8, 5.9 and 3.0 cc. for the high, normal and low protein diet. In the case of organic acids, there was a daily as well as an individual variation which could not be explained by dietary changes. The average values with creatinine correction were 7.9, 6.7 and 7.1 cc. N/10 acid for the high, normal and low protein diets. These values are in close agreement with those reported by Van Slyke and Palmer (27) and McLaughlin and Blunt (28). In the study made on normal young men, the former gave an average value of 8.2 cc. (uncorrected) per kilo. The latter found a range of 6.8 to 10.0 cc. (uncorrected) of N/10 acid per kilo. per day in a study made on a group of university women. They also stated that the daily variation was great even when the diet was constant and further a low protein diet had no influence on the organic acid excretion.

Lactic acid was very constant in all the subjects throughout this investigation with an average value of 0.3 cc. N/10 acid per kilo. of body weight. The amount of protein in the diet apparently had little or no effect on the excretion of lactic acid. We were unable to find any references with direct bearing on this problem as most of the work reported dealt with pathological subjects or with the influence of exercise rather than with diet on normal subjects. Clausen (29) in his study made on a series of pathological patients gave an average of 4 normal subjects as 5.4 mg. of lactic acid excreted per kilo. of body weight per day which is equivalent to 0.6 cc. N/10 acid. This higher value might be due to the condition of his subjects as there seems to be a question in the mind of the author. Using a different technique, Ishihara (30) gave as normal value in man 8 mgs. per 100 cc. of urine and Hill and his associates (31), in their study entitled Muscular Exercise 1, Lactic Acid, and the Supply and Utilization of Oxygen, observed a variation from 3 to 14 mgs. of lactic acid per 100 cc.

Summary

1. A study of the influence of the protein level on basal metabolism and blood and urine chemistry was conducted on 6 normal adult women varying in age from 17 to 36 years. The subjects received for 5 weeks a high protein diet containing 2 gm. of protein, and sufficient carbohydrate and fat to make 40 calories, per kilo. of body weight. After an interval

of 3 weeks, during which time the protein content of the diet was decreased until a level of 0.6 gm. was reached at a constant caloric value of 40, the subjects remained on this diet for 4 weeks. About a month later the same subjects were placed on a normal diet for 2 weeks during which time they were allowed to choose their own food as well as the amount.

2. No marked difference was found in the basal metabolic rate of the subjects during the three periods. A slight increase was observed during the normal diet which might be due to an improved physical condition following a summer vacation or to the difference in the temperature of the season.

3. An increase was found in the total non-protein nitrogen, urea nitrogen and creatine of the blood during the high protein period. No change was observed in uric acid, creatinine and calcium. There was a slight increase in the sugar and a marked increase in the blood lactic acid during the low protein period.

4. No difference was found in blood pressure.

5. As was expected, the total urinary nitrogen, urea nitrogen, ammonia nitrogen, uric acid and creatine varied directly with the protein intake. Creatinine remained constant throughout the investigation.

6. Total titratable acidity in the urine varied with the ammonia and hence with the protein intake. There was both a daily and an individual variation in organic acids. Lactic acid remained constant throughout the different periods.

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